BIOC-416

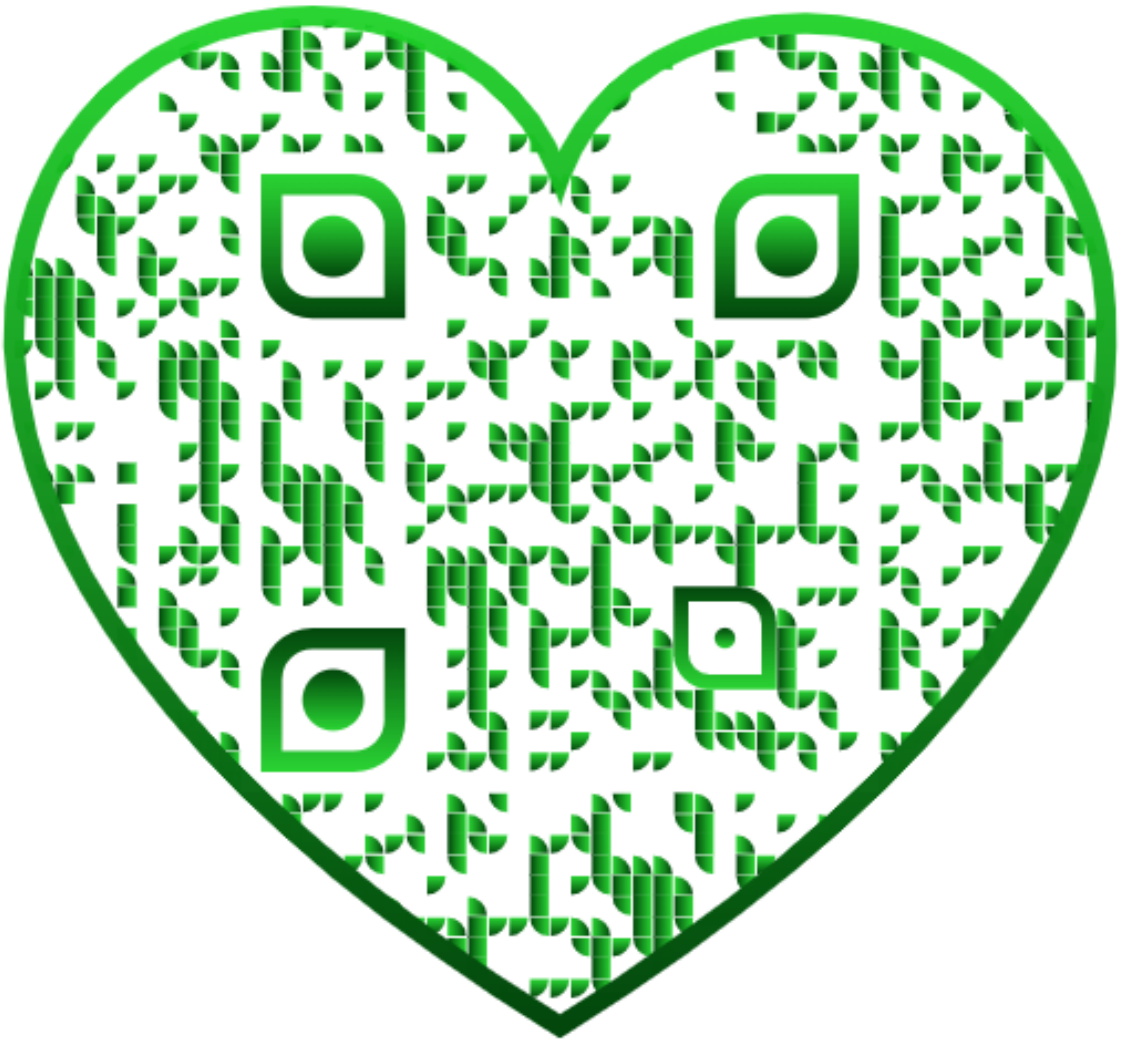
KING ABDULAZIZ UNIVERSITY

BIOCHEMISTRY DEPARTMENT

CLINICAL BIOCHEMISRY [LAB MANUAL[

Done by: Lecturer/Sharifa A. Al-Ghamdi

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari



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LAB-1

Introduction to Clinical Laboratories

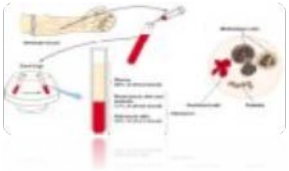
When a person is ill, diagnosis begins with physical examination by a doctor. It may not possible to diagnose a disease only on the basis of physical examination. There are various diagnostic tests to confirm a suspected diagnosis. The clinical/pathological laboratory tests are extremely useful to find out the causes of disease.

The functional components of the clinical laboratory are:

1) Clinical pathology
2) Hematology
3) Clinical biochemistry
4) Clinical microbiology
5) Serology
6) Blood bank
7) Histology and cytology

Functions:

Clinical biochemistry deals with the biochemistry laboratory applications to find the cause of a disease as well as the severity of diseases of many organs such as liver, stomach, heart, kidneys, brain as well as the endocrine disorders and related status of acid-base balance of the body.



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The function of clinical laboratory is to perform qualitative and quantitative analysis on body fluids such as blood (serum or plasma), urine, feces, cerebrospinal fluid (CSF), other body fluids, tissues or calculi.

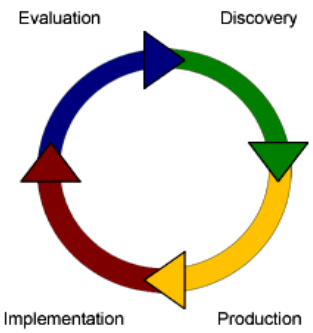
- **The clinical biochemistry tests**, in relation to various clinical conditions can:

1) Reveal the cause of the disease
2) Screen easy diagnosis
3) Suggest effective treatment
4) Assist in monitoring progress of pathological condition
5) Help in assessing response to treatment

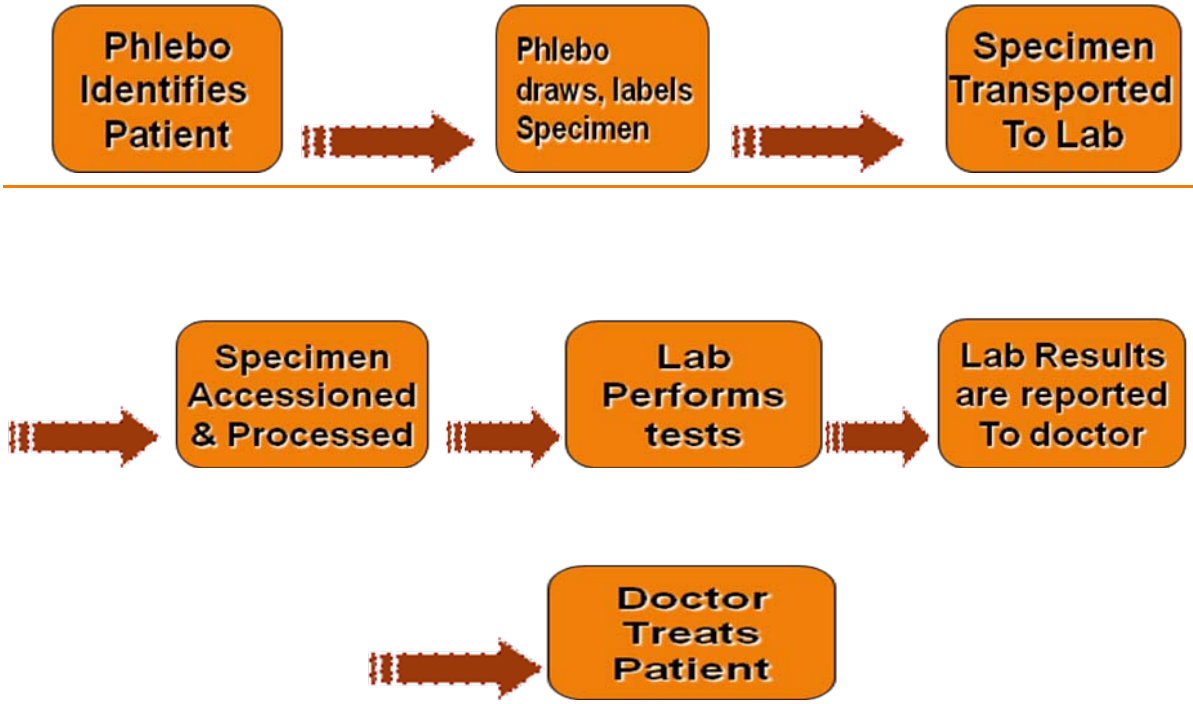
Disinfection:

Chlorine (Sodium hypochlorite) is an universal disinfectant which is active against all microorganisms. Normally, it is available as sodium hypochlorite. It is a strong oxidizing agent. A general all-purpose laboratory disinfectant solution of sodium hypochlorite should have a concentration of 1g/L.

Laboratory work flow cycle:



The flow cycle includes the entire steps of laboratory test, starting from test ordering by a doctor until reporting the results. See the figure



Phlebotomy equipments:

The phlebotomist ,the technician who collects blood, should be trained to:

1) Prepare specimen collection material
2) Instruct patient appropriately
3) Collect, preserve and transport specimen carefully
4) Separate serum or plasma properly
5) Maintain proper record of collection
6) Handle the specimen carefully

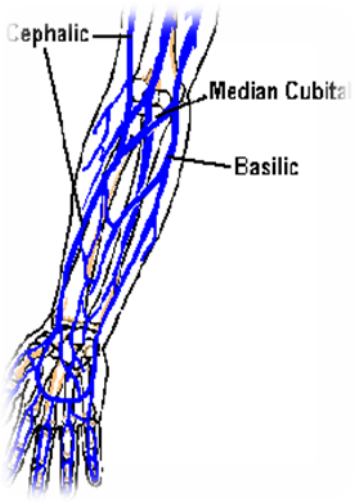
7) Analyze the specimen accurately
8) Maintain proper record of reports
9) Work with appropriate safety precautions

The phlebotomy equipments: Following material should be readily available in the specimen collection section:

1) Disposable syringes or vacutainer systems
2) Disposable lancets
3) Gauze pads or adsorbent cotton
4) Tourniquet
5) Alcohol swap
6) Waste container

Blood collection:

Selecting vein site:



For most venipuncture procedure on adults vein located in the arm are used. The median cubital vein is the one used for the patient. If the venipuncture of this vein is unsuccessful, one of cephalic or basilic veins may be used. The blood however usually flows more slowly from these veins.

Note: For the determination of blood pH, PCo2, PO2 and bicarbonate, arterial blood is used. It is usually performed by physicians.

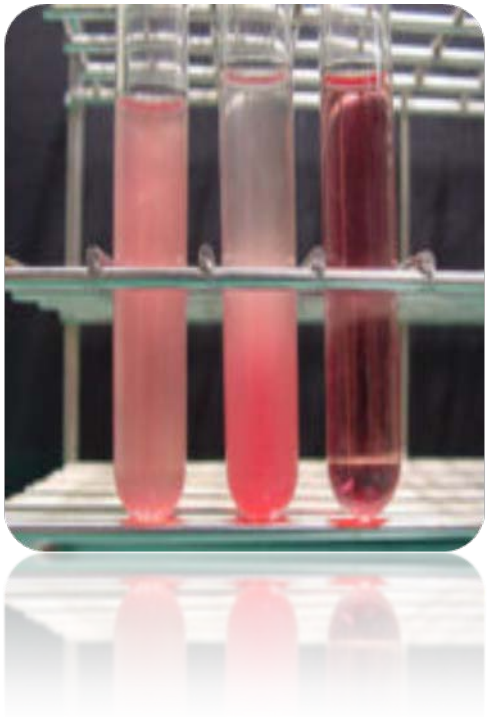
Specimen rejection criteria:

1- Specimen improperly labeled or unlabeled
2- Specimen improperly collected or preserved

3- Specimen submitted without properly completed request form

4- If separated plasma or serum is grossly hemolyzd.

Hemolysis of blood:



Hemolysis means liberation of hemoglobin from RBCs. Due to hemolysis, plasma or serum assumes pink to red color. It is important to avoid hemolysis during sampling, transporting and storage (too hot or too cold)because hemolysis causes changes in measurement of a number of analysis such as:

1- Serum K

2- Serum in.org P.

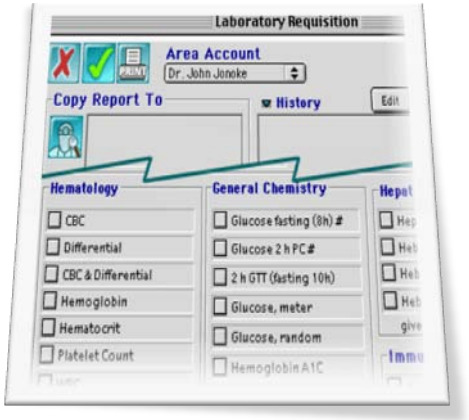
3- SGOT

4- SLDH

5- Acid phosphatase

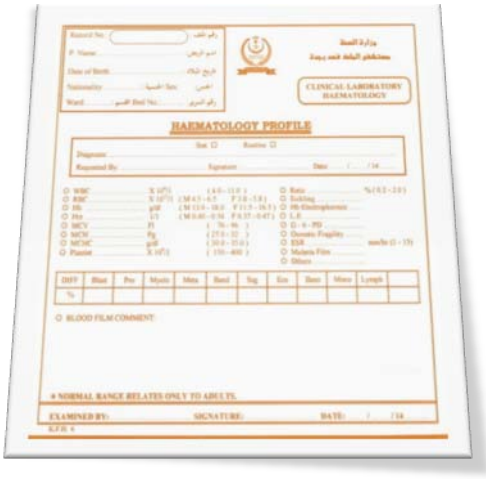
Lab request:

The order or lab request contains a list of tests to be performed on one or more patient specimen, for example blood or urine. Each lab has its specific request that contains tests that performed in that lab only i.e. chemistry request, hematology request…etc.



The following items should be included on the lab requisition:

1. Full name: middle name should be included to avoid confusion in the event that there is another patient with the same first and last name.
2. Location: inpatient, room, unit, outpatient, address.
3. Patient's identification number: this identification can be very useful for instance in the blood bank.
4. Patient age and sex: in evaluating laboratory results, the reference values may differ for age and sex; disease prevalence may be age- or sex-linked.
5. Name(s) of the physician(s): name all of the physicians on the case; "panic values" should be called to the attention of the physician ordering the test; a physician may have some specific test guidelines for his patients.
6. Name of the test and the source: reference values may be different for the different biologic specimens (e.g., serum and CSF glucose
7. Possible diagnosis: essential for evaluating laboratory results and selecting appropriate methodology; (media selection in microbiology).
8. The date and time the test is to be done: some tests must be scheduled by the laboratory; patient preparation and diet regulations need to be considered.
9. Special notation: provide relevant information to assist the laboratory--e.g., medications taken; for hormone assay, the point in the menstrual cycle when the specimen was obtained.



Specimen collection:

There are many factors to consider when collecting lab specimens; and prior to diagnostic tests. Preparation of the patient prior to the test or diagnostic measure is vitally important to the results of the test. Many laboratory tests and diagnostic tests do not require any extensive preparation. The amount of the sample needed depends upon many factors. Each lab is different in the amount of blood or other body fluid or tissue required to perform the analysis. Generally speaking, if the blood is run using modern automated analyzers, the amount of blood may be 10 ml or less for each test. If the tests are run individually, or if the tests are complicated, larger quantities of blood may be needed.

Blood collection tubes:

The tubes are covered with a color-coded plastic cap.

They often include additives that mix with the blood when collected, and the color of the tube's plastic cap indicates which additives that tube contains.

The tubes may contain additional substances that preserve the blood for processing in clinical laboratory.

Using the wrong tube may therefore make the blood unusable.

-Plasma Separating Tubes(PST)

LAVENDER

-It contains EDTA which is a strong anticoagulant.

-It is used mainly foe hematology studies.

-It must be inverted several times after collection

LIGHT BLUE

-sodium citrate.

-coagulation (clotting) studies.

-must be completely filled

-must be inverted immediately after filling

GREEN

-sodium or lithium heparin

-for tests requiring whole blood or plasma such as ammonia

-must be inverted several times after collection

Black

-Contains sodium citrate

-Used for ESR

-must be inverted several times after collection

GRAY

-Sodium Fluoride +potassium oxalate.

-It is used for measuring glucose levels.

-must be inverted several times after collection

ROYAL BLUE

-heparin or Na EDTA anticoagulants

-Tube is designed to contain no contaminating metals

-Trace element and toxicology studies

YELLOW

-This tube is used for certain reference tests requiring whole blood.

- It contains ACD (acid-citrate-dextrose) as the anticoagulant.

- It is also used for blood cultures.

Serum Separating Tubes(SST)

Red (Plain tube)

-contains no additives.

-Tests for antibodies and drugs often require these.

Gold

-These contain particles that cause blood to clot quickly, as well as a gel to separate blood cells from serum.

Blood:

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments.

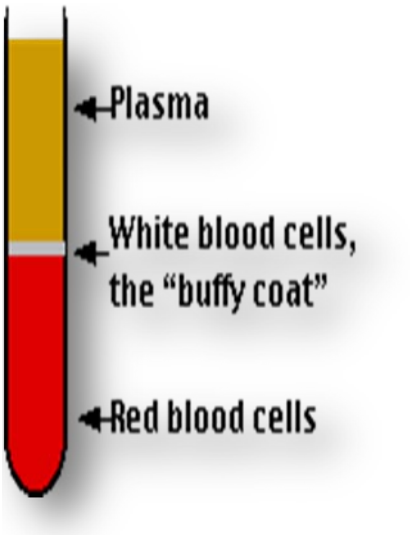
-Red blood cells(RBCs)

-White blood cells(WBCs)

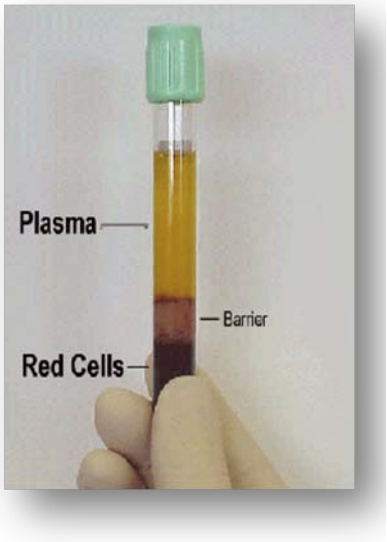
-Platelets

-Five kinds of Leukocytes (lymphocytes, monocytes,neutrophils,eosinophils,basophils)

-After centrifugation of blood, the blood separate into three layers(see the figure)



Blood plasma:



-Plasma is the liquid component of blood.

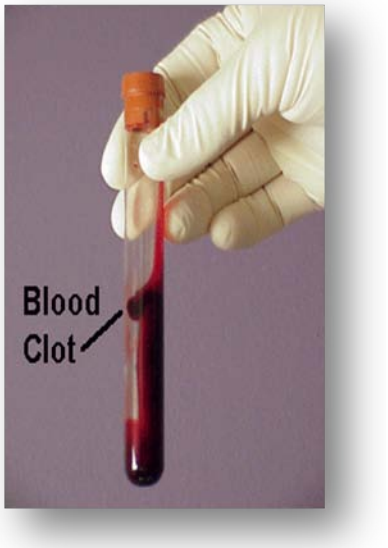
-It is mainly composed of water, blood proteins and inorganic electrolytes.

-Roughly 92% water, mixed with organic and inorganic-substances.

- The most abundant plasma solute is the plasma protein, of which there are three groups: albumin, globulins, and fibrinogen.

Blood clot:

-When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot.



-One of the normal components of plasma is a soluble plasma protein called fibrinogen.

-On standing, this protein will be converted to insoluble substance called fibrin >>>> this occurrence is referred to as blood coagulation or clotting.

-The clot contains coagulation proteins, platelets, and entrapped red and white blood cells.

Blood serum:

Serum is the same as plasma except that clotting factors (such as fibrin) have been removed.

-For many biochemical laboratory tests, plasma and blood serum can be used interchangeably. Serum resembles plasma in composition but lacks the coagulation factors.

- It is obtained by letting a blood specimen clot prior to centrifugation.

Procedure of Plasma Preparation:

1-Draw blood from patient. Select vacutainer with an appropriate anticoagulant.

2- Mix well with anticoagulant.

3- Allow to stand for 10min.

4- Centrifuge the sample to speed separation and affect a greater packing of cells.

5- The supernatant is the plasma which can be now collected for testing purposes or stored (-20C to -80C) for subsequent analysis or use.

Procedure of Serum preparation:

1- Draw blood from patient. Select vacutainer with NO anticoagulant.

2- Allow to stand for 20-30min for clot formation.

3- Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells will separate from clean serum and settle to the bottom of the vessel.

4- The supernatant is the serum which can be now collected by dropper or pipette for testing purposes or stored (-20C to -80C) for subsequent analysis or use.

References:

Springhouse Handbook of Diagnostic Tests, 2nd edition, Springhouse Co., 1999.

Brunner, L.S. & Suddarth, D.S., The Lippincott Manual of Nursing Practice, JB Lippincott, 1999.

Buckingham, Alan, Arterial Blood Gases Made Simple, Nursing Life, Nov/Dec 1994.

Diagnostic Tests Handbook, Springhouse, Penn. Intermed Communications, 1994.

Fischbach, F., A Manual of Laboratory diagnostic Tests, Philadelphia, Lippincott, 1998.

Kee, Joyce LeFever; Laboratory and Diagnostic Tests with Nursing Implications, Appleton-Century-Crofts, 1991.

www.wikipedia.org

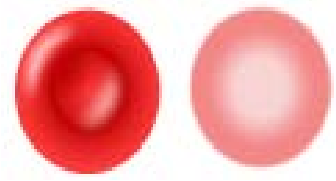
LAB-2

Reticulocyte (Retic.)Count

Staining:

- It is a biochemical technique of adding a dye to a substrate to qualify or quantify the presence of a specific compound.
- Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing with the aid of different microscopes.
- Stains may be used to define and examine bulk tissues or classify different blood cells. Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas.

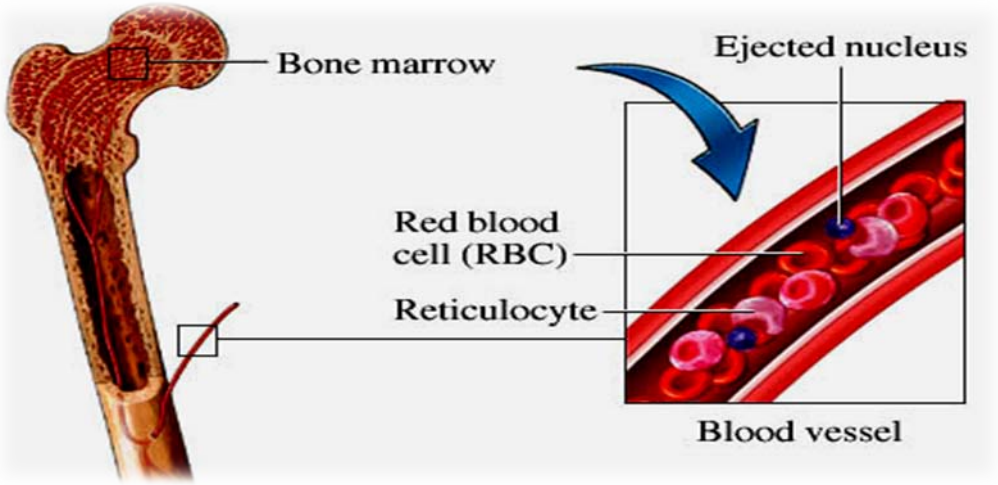
-For retic. Count new methylene blue (NMB) dye is used.



Reticulocyte is immature RBCs. typically composing about 1% of the red cells in the human body. Reticulocytes develop and mature in the red bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. Like mature red blood cells, reticulocytes do not have a cell nucleus. They are called reticulocytes because of a reticular (mesh-like) network of rRNA that becomes visible under a microscope with certain stains such as methylene blue.

Reticulocyte count:

The reticulocyte count is blood test performed to assess the percentage of circulating red blood cells that are in the reticulocyte stage. A reticulocyte count is usually performed when patients are evaluated for anemia and response to its treatment. It is sometimes called a retic count.The normal range of values for reticulocytes in the blood depends on the clinical situation and the lab, but broadly speaking is 0.5% to 1.5%. However, if a person has anaemia, their reticulocyte percentage should be higher than "normal" if the bone marrow's ability to produce new blood cells remains intact.



Diagnosis:

A reticulocyte count provides information about the rate at which the bone marrow is producing red cells. A normal count means that the production is adequate; a decreased count means it is not. This information helps determine whether a lack of red cells in an anemic person is caused by a bone marrow problem, by excessive bleeding, or by red cell destruction.

Monitoring:

The test is also used to monitor the response of bone marrow to treatment for anemia. The reticulocyte count rises within days if the treatment is successful. It is also used following bone marrow transplant to evaluate the new marrow's cell production.

Principal:

Ribosomes react with NEW METHYLENE BLUE to form a blue precipitate of granules or filaments. The reaction takes place only in vitally stained unfixed preparations. Cells containing the blue precipitate or filaments are expressed as a percentage of the red cells counted.

Equipments and reagents:

- 12 x 75 mm disposable tube
- Glass slides
- Disposable pipettes
- Microscope
- New Methylene Blue

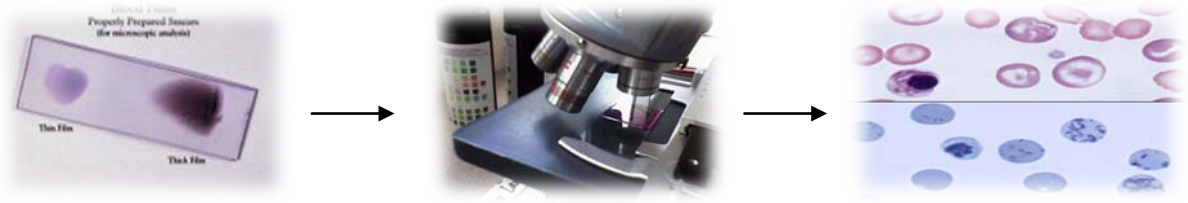
SPECIMEN:

-Whole blood collected in EDTA tube.

Procedure:

1. Mix equal volume of whole blood and New Methylene Blue in a glass or plastic tubes.
2. Incubate the mixture at 37° C for 20 minutes.
3. Resuspend the cells by gentle mixing.
4. Make a blood film of the mixture.
5. Examine the film under oil immersion.
6. Count one thousand RBCs while tallying the number of reticulocytes.

-Reticulocytes appear as cells containing dark blue granules or a blue network. The number of reticulocytes is reported as a percentage of the total red cells.



Results:

In a healthy patient, the reticulocyte percentage is very stable. When the number of RBCs decreases (thus the hematocrit decreases), the percentage of reticulocytes may appear increased compared to the overall number of RBCs. In order to get a more accurate assessment of bone marrow function, the calculated reticulocyte percentage (%) is often corrected with a calculation called a corrected reticulocyte count or a reticulocyte index (RI). This calculation compares the patient's hematocrit with a normal hematocrit value.

Reticulocyte (%) = [Number of Reticulocytes / Number of Red Blood Cells] X 100

Reticulocyte Index = Reticulocyte count (%) X [Measured hematocrit / Normal hematocrit]

Normal results:

Adults have reticulocyte counts of 0.5-2.5%. Women and children usually have higher reticulocyte counts than men.

Abnormal results:

A low reticulocyte count indicates that the bone marrow is not producing a normal number of red blood cells. Low production may be caused by a lack of vitamin B_{12}, folic acid, or iron in the diet; or by an illness affecting the bone marrow (for example, cancer). Further tests are needed to diagnose the specific cause.

The reticulocyte count rises when the bone marrow makes more red cells in response to blood loss or treatment of anemia.

References:

- www.wikipedia.org

- www.medicaldesign.com

- Rowan, R. M., et al. "The Reticulocyte Count: Progress Towards the Resurrection of a Useful Clinical Test." Clinical and Laboratory Haematology 18, no. 1 (1996): 3-8.

Lab Report

King Abdul-Aziz University
College of Science
Biochemistry Department
Clinical Biochemistry Course

Patient Name:	**Age / Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

DESCRIPTION	RESULT	UNIT	REFERNCE RANGE
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EXAMINED BY:
DATE/ TIME:

LAB-3

Liver Function Tests (LFTs)

ALT&AST

The liver is in the upper right part of the abdomen.

The functions of the liver include:

1-storing glycogen (fuel for the body) which is made from sugars.

2-helping to process fats and proteins from digested food.

3-making proteins that are essential for blood to clot (clotting factors).

4-processing many medicines which you may take.

5- helping to remove poisons and toxins from the body.

Liver function tests:

-LFTs are group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.

-As the liver performs it's various functions it makes a number of chemicals that pass into the bloodstream and bile. Various liver disorders alter the blood level of these chemicals. Some of these chemicals can be measured in a blood sample. Some tests that are commonly done on a blood sample are called 'LFTs' (liver function tests).

Liver function tests can be classified as:

a. Tests of excretion by the liver.
b. Evaluation of synthesis in liver.
c. Evaluation of enzyme activity.

Liver function tests are most often employed to determine:

i. The presence of liver disease.
ii. The type of liver disease.
iii. The extent and progression of liver disease.

Note: The diagnosis of liver disease depends upon a complete history, complete physical examination, and evaluation of liver function tests and further invasive and noninvasive tests. These usually measure the following:

- **Alanine Aminotransferase (ALT)=SGPT:**

  -ALT is the enzyme produced within the cells of the liver. The level of ALT abnormality is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT. ALT is the most sensitive marker for liver cell damage.

- Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders.
- Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders.
- In acute inflammatory conditions of the liver, ALT elevated higher than AST. (more specific than AST).

- **Aspartate Aminotransferase (AST)=SGOT:**

-This enzyme also reflects damage to the hepatic cell. It is less specific for liver disease. It may be elevated and other conditions such as a myocardial infarct (heart attack). Although AST is not a specific for liver as the ALT, ratios between ALT and AST are useful to physicians in assessing the etiology of liver enzyme abnormalities.

- **Alkaline Phosphatase (ALP):**

-Alkaline phosphatase is an enzyme, which is associated with the biliary tract. It is not specific to the biliary tract. It is also found in bone and the placenta.

-If the alkaline phosphatase is elevated, biliary tract damage and inflammation should be considered. One of the more common methods to assess the etiology of the elevated alkaline phosphatase is to determine whether the GGT is elevated or whether other function tests are abnormal (such as bilirubin)

-Alkaline phosphatase may be elevated in primary biliary cirrhosis, alcoholic hepatitis---

- **Gamma Glutamic Transpeptidase (GGT):**

-This enzyme is also produced by the bile ducts. However, it is not very specific to the liver or bile ducts. It is used often times to confirm that the alkaline phosphatase is of the hepatic etiology. Medications commonly cause GGT to be elevated. Liver toxins such as alcohol can cause increases in the GGT.

- **Other tests include**.
    1- Blood clotting tests
    2- Total proteins.

3- Immunological studies

4- Prothrombin time (PT)

5- Liver biopsy, ultrasound scan, other types of scan, etc, may be needed to clarify the cause of a liver disorder, and/or to monitor its progress.

Lab practices:

-Collect blood and prepare serum in an appropriate tube.

-Measure ALT and AST levels.

-Compare the results to the normal values.

Assay for Enzyme Activity:

- Alanine + α-Ketoglutarate $\xrightarrow{ALT}$ Glutamate + Pyruvate
- Pyruvate + NADH + H $\xrightarrow{LDH}$ Lactate + NAD

- The rate of decrease in conc. of NADH, measured photometrically, is proportional to the catalytic con. of ALT present in the sample.

Reference Range:

Normal: 6 – 37 U/L.

Interfering Factors:

- Many drugs may cause falsely increased and decreased ALT levels.
- Therapeutic heparin increases ALT.
- Hemolysed blood increases ALT.

Lab Report

King Abdul-Aziz University
College of Science
Biochemistry Department
Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

DESCRIPTION	RESULT	UNIT	REFERNCE RANGE
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EXAMINED BY:
DATE/ TIME:

LAB-4

Liver Function Tests (LFTs)

Measurement of Serum Bilirubin (Total, direct &indirect)

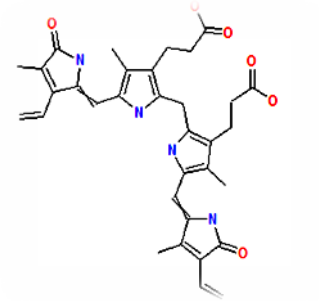
Bilirubin is a yellow breakdown product of normal heme catabolism.

Its levels are elevated in certain diseases and it is responsible for the yellow color of bruises and the brown color of feces.

Bilirubin reduction in the gut leads to a product called urobilinogen, which is excreted in urine

Chemistry:

Bilirubin consists of an open chain of four pyrroles (*tetrapyrrole*);by contrast, the heme molecule is a ring of four pyrroles, called porphyrin.



-Like other pigments, bilirubin changes its conformation when exposed to light.

-This is used in the phototherapy of jaundiced newborns: the illuminated version of bilirubin is more soluble than the unilluminated version.

Production and Metabolism

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

I-Production:

- After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial (RE) system, particularly in the liver, spleen and in the bone marrow.
- This releases hemoglobin which destroyed to the heme and the protein portion (globin).
- The globin parts are turned into amino acids.
- Iron is removed from the heme molecule, and the porphyrin ring is opened to form bilirubin.
- The body usually produces about 300 mg of bilirubin per day as a breakdown product of heme. About 80% arises from red cells with the remainder coming from red cell precursors destroyed in the bone marrow (‘ineffective erythropoiesis’), and from other heme proteins such as myoglobin and the cytochromes.

II-Transport in plasma and hepatic uptake:

Bilirubin is insoluble in water and is carried in plasma bound to albumin, and thus is not filtered at the glomerulus unless there is glomerular proteinuria.

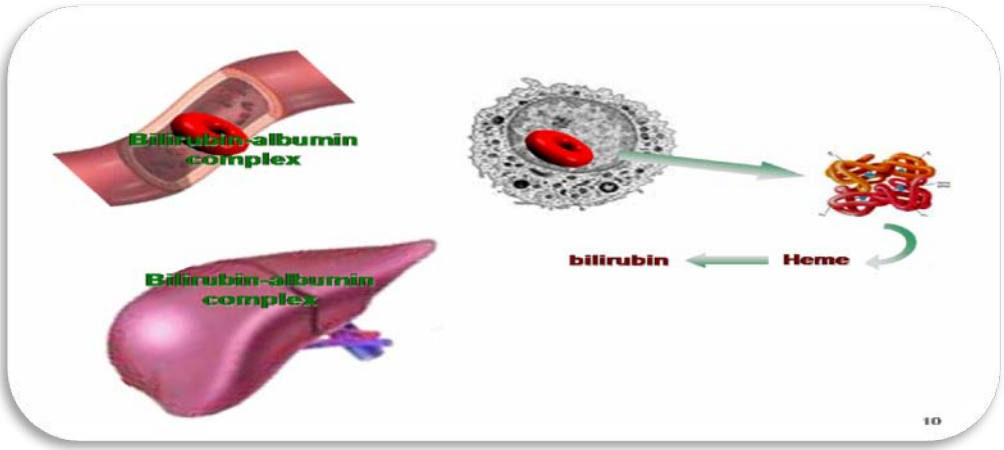
On reaching the liver, the bilirubin is taken into the hepatocyte by specific carrier mechanism

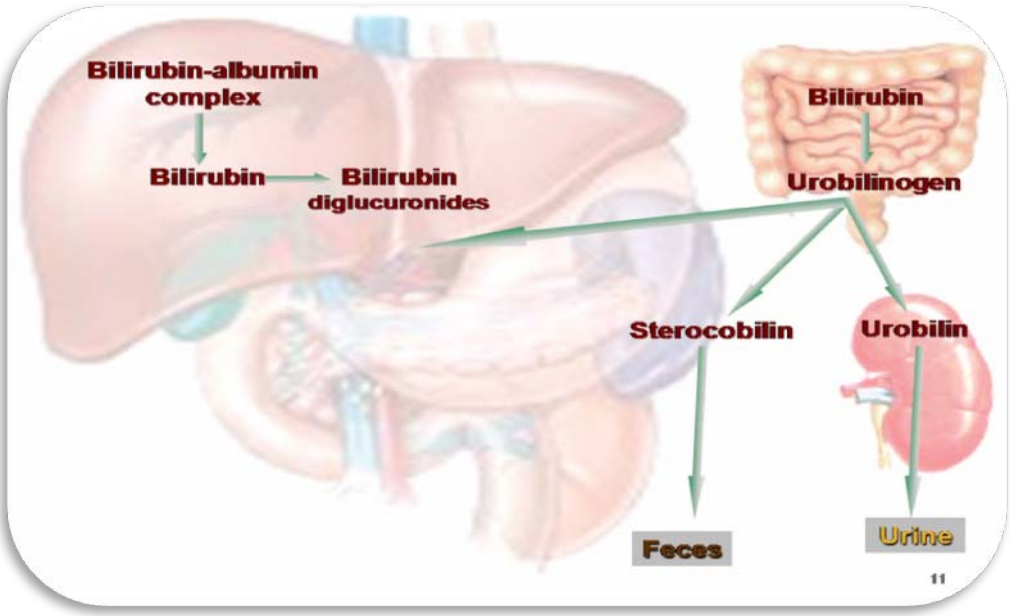
III. Conjugation of bilirubin and secretion into bile:

- In the endoplasmic reticulum of the hepatocyte, the enzyme bilirubin UDP-glucuronyltransferase conjugates bilirubin with glucouronic acid to produce bilirubin diglucuronides which are water soluble and readily transported into bile.

IV. Further metabolism of bilirubin in the gut:

- In the intestine, glucouronic acid is removed by bacteria.
- The resulting Bilirubin is converted to urobilinogen.
- Most of urobilinogen is oxidized by intestinal bacteria to sterocobilin, which gives faeces its brown colour.
- However, some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
- A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle in which it is taken by the liver, and then re-exerted into the bile.
- The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and exerted, giving urine its colour.
- If the liver's function is impaired, or biliary drainage blocked, some of the conjugated bilirubin appears in the urine, turning it dark.





Measurements of plasma bilirubin:

- Serum bilirubin concentration depends on the rate of removal of bilirubin from destruction of hemoglobin.
- A bilirubin test measures the amount of bilirubin in a blood sample.
- Total and direct bilirubin levels can be measured from the blood, but indirect bilirubin is calculated from the total and direct bilirubin.

Types of Bilirubin:

Bilirubin is present in plasma as:

- Indirect Bilirubin (**unconjugated bilirubin**)
- Direct Bilirubin (**conjugated bilirubin**)

Jaundice:

- Is a term used in clinical medicine to describe a condition in which the skin and sclera appear yellow.

Classification of the causes of Jaundice:

1. Prehepatic jaundice
2. Hepatic jaundice
3. Posthepatic jaundice

Prehepatic jaundice

- Results when excessive amount of bilirubin is presented to liver for metabolism, such as in hemolytic anemia.
- Characterized by **unconjucated hyperbilirubinemia.**
  Bilirubin not appear in urine. **Why?**

Hepatic jaundice

Results from:

- Impaired cellular uptake.
- Defective conjugation.
- Abnormal secretion of bilirubin by the liver cell.

Posthepatic jaundice

- Results from Impaired excretion of bilirubin caused by mechanical obstruction of the flow of bile into intestine.
- This may be due to gallstones or a tumor.
- Rise in the serum **conjugated bilirubin** level and stool becomes clay-colored. **Why?**
- Conjugated bilirubin appears in the urine and the urine urobilinogen levels decrease.
- Too much bilirubin (hyperbilirubinemia) in a newborn baby can cause brain damage, hearing loss, problems with the muscles that move the eye, physical abnormalities, and even death.
- Therefore, some babies who develop jaundice may be treated with special lights (phototherapy) or a blood transfusion to reduce their bilirubin levels.
- Determining a neonatal bilirubin level is considered standard medical care.

Lab practices:

-Collect blood and prepare serum in an appropriate tube.

-Measure TBil, direct Bil, and indirect Bil levels.

-Compare the results to the normal values.

Principle:

Bilirubin in the presence of a Sulphanilic Acid diazonium salt forms a red coloured azo compound in alkaline solutions. The total and direct bilirubin in serum is determined using the method of Jendrassik and Grof by coupling it with diazotised sulphanilic Acid after the addition of caffeine, sodium benzoate.

Reagent Concentration:

Reagent 1. Sulphanic acid solution: Sulphanilic acid 30mmol/L

Reagent 2. Sodium nitrite solution: Sodium nitrite 50mmol/L

Reagent 3. Caffeine solution: Caffeine 100mmol/L

Samples:

For use with serum or plasma samples

Haemolysis interferes with the test.

Do not expose sample to sunlight or other light.

Samples can be stored for up to 3 months at –20 C, 4 days at 2-8 C and 1 day at 15-25 C

Safety precautions:

For in vitro diagnostic use only. Do not pipette by mouth.

Exercise the normal precautions required for handling laboratory reagents.

Procedure:

Wavelength: Hg 546 (540nm)

Temperature: +20 to +25ºC

Cuvette: 1cm light path

Zero adjustment: Against sample blank

	Total	Direct	Blank
R1	100 µl	100 µl	100 µl
R2	25 µl	25 µl	----
Saline	----	1ml	1ml
R3	1ml	----	----
Sample	100 µl	100 µl	100 µl

Mix. Let stand for 5 minutes at +20-+25ºC.

Read absorbance of total and direct samples

Calculation:

Total Bilirubin (mg/dl)=Absorbance tube Total x 17.5

Direct Bilirubin(mg/dl)=Absorbance tube Direct x 17.5

(mg/dl) x 17.1 = µmol/L

Linearity:

The method is linear up to 20mg/dl (342µmol/l).

In case of higher results, dilute sample 1:2 with NaCl 0.9% solution and repeat test. Multiply result by 2.

Normal Values:

Serum:

Total Bilirubin up to 1.1mg/dl (18.8µmol/l)

Direct Bilirubin up to 0.25mg/dl (4.3µmol/l)

References:

Jendrassik, L. et al. Biochem. Z. *297*:81 (1938)

Malloy, H.T. and Evelyn, K.A., J. Biol. Chem. 119, 481(1937)

Matimek, R.G. Clin Chem. Acta, 13 161 (1966)

Lab Report

King Abdul-Aziz University
College of Science
Biochemistry Department
Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

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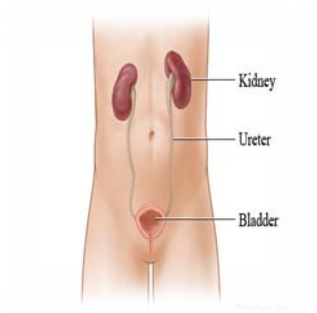
LAB-5

Renal Function Tests

Measurement of Serum BUN



The kidneys, the body's natural filtration system, perform many vital functions, including removing metabolic waste products from the bloodstream, regulating the body's water balance, and maintaining the pH (acidity/alkalinity) of the body's fluids. Kidney function tests help to determine if the kidneys are performing their tasks adequately.

Renal function tests (RFTs):

Kidney function tests is a number of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys can help determine the cause and extent of kidney dysfunction. These tests are done on urine samples, as well as on blood samples.

Blood tests:

There are several blood tests that can aid in evaluating kidney function. These include:

1-Blood urea nitrogen test (BUN).

2-Creatinine test.

3-Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

I-Measurement of BUN:

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

- Urea is a by-product of protein metabolism. This waste product is formed in the liver, then filtered from the blood and excreted in the urine by the kidneys.
- The BUN test measures the amount of nitrogen contained in the urea.
- High BUN levels can indicate kidney dysfunction, but because blood urea nitrogen is also affected by protein intake and liver function, the test is usually done in conjunction with a blood creatinine, a more specific indicator of kidney function.

A high BUN value can mean

1-kidney disease

2-Blockage of the urinary tract (by a kidney stone or tumor)

3- Low blood flow to the kidneys caused by dehydration or heart failure.

4-Many medicines may cause a high BUN.

5-A high BUN value may be caused by a high-protein diet, tissue damage (such as from severe burns), or from bleeding in the gastrointestinal tract.

A low BUN value may be caused by

1- A diet very low in protein, malnutrition, or severe liver damage.

2-Women and children may have lower BUN levels than men because of how their bodies break down protein.

Lab practices:

-Collect blood in an appropriate tube.

-Run BUN test.

-Compare the results to the normal values.

Principal:

Urea reacts with diacetyl-monoxime in hot acidicmedium and in the presence of thiosemicarbazide and ferric ions to form a pink colored compound.

Requirements:

1- Test tubes
2- 10 ml pipettes
3- 0.1 ml serologic pipette
4- Measuring cylinder
5- Water-bath
6- Stopwatch
7- Spectrophotometer

Samples:

Serum or heparinized plasma

Preparation of reagents:

1- Reagent1: (DMR) it contains 0.2 g/dl diacetyl monoxime in DW. The reagent is stable at room temp. for one year.
2- Reagent 2: (TSC) it contains 40 mg/dl thiosemicarbazidein DW. The reagent is stable at room temp. for 6 months.
3- Reagent 3: (Acid) it contains 60 ml of conc. sulphuric acid, 1o ml orthophosphoric acid and 10 ml of 1 gm/dl ferric chloride in orthophosphoric acid in one liter of the reagent prepared in DW

The reagent is stable at room temp for one year.

4- Urea nitrogen standard: 20 mg/dl: it contains 42.8 mg of urea in 100 ml of saturated benzoic acid.

This standard is stable for one year when refrigerated.

Preparation of working reagent:

It is prepared fresh by mixing one part of reagent 1 + one part of reagent 2 +two parts of reagent 3. This reagent should be prepared fresh.

Procedure:

Pipette in the tubes labeled as follows:

	Test	Standard	Blank
WR(ml)	5	5	5
Serum(µl)	50	-----	-----
Std(µl)	-----	50	------
DW(µl)	-----	-----	50

Mix well and place in boiling water bath for exactly 15 minutes.

Cool immediately and after 5 minutes read the absorbance at 520 nm.

Calculations:

BUN mg/dl= O.D. test/O.D. standard X20

Linearity:

This method is linear up to 50 mg/dl

Normal Values:

- Birth-one year: 4-16 mg/dl
- 1-40 years: 7-12 mg/dl -Gradual slight increase occurs over 40y

- Panic range: BUN>100 mg/dl

References:

- Marsh, W., Fingerhut, B. and Miller, H. (1965), Clin. Chem. 11, 624.
- Fearon, W. (1939), Bioch. J., 33, 902.
- Wybenga, D.R. (1971), Clin. Chem. 17, 891.

Lab Report

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Patient Name:	**Age / Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

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DATE/ TIME:

LAB-6

Renal Function Tests

Measurement of Serum Creatinine &Creatinine Clearance

Creatinine test:

This test measures blood levels of creatinine, a by-product of muscle energy metabolism that, like urea, is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on an individual's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine is a more sensitive indication of impaired kidney function than the BUN.

BUN: Creatinine ratio:

A BUN test may be done with a blood creatinine test. Blood urea nitrogen (BUN) and creatinine tests can be used together to find the BUN-to-creatinine ratio (BUN: creatinine).

BUN-to-creatinine ratio 10:1–20:1

High BUN-to-creatinine ratio occur with sudden (acute) kidney failure. A blockage in the urinary tract (such as a kidney stone) can cause a high BUN-to-creatinine ratio.

A very high BUN-to-creatinine ratio may be caused by bleeding in the digestive tract or respiratory tract.

A low BUN-to-creatinine ratio may be caused by a diet low in protein, a severe muscle injury and others.

Creatinine clearance:

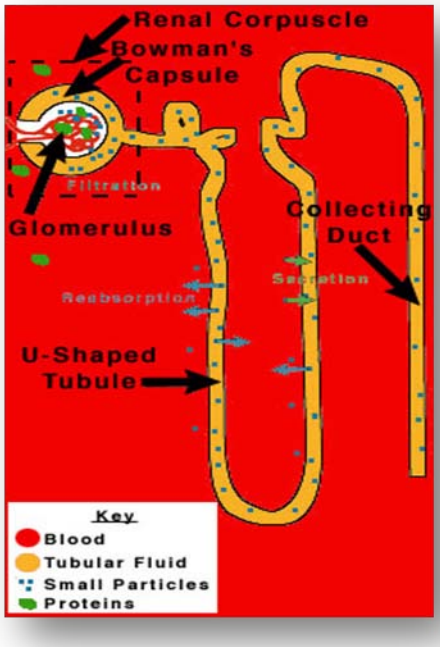
Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

In general, creatinine clearance is the removal of creatinine from the body.

More accuratly, creatinine clearance (ClCr) is the volume of blood plasma?? that is cleared of creatinine per unit time.

Clinically, creatinine clearance is a useful measure for estimating the glomerular filtration rate (GFR) of the kidney.

The result of this test is an important parameter used in assessing excretory function of the kidneys.



- Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function. A rise in blood creatinine levels is observed only with marked damage to functioning nephrons. Therefore this test is not suitable for detecting early stage kidney disease.
- A better estimation of kidney function is given by the creatinine clearance test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, and weight without a 24 hour urine collection
- Some laboratories will calculate the CrC if written on the pathology request form; and, the necessary age, sex, and weight are included in the patient information.

Cockroft-Gault formula:

- A commonly used marker for actual creatinine clearance is the Cockroft-Gault formula, which employs creatinine measurements

and a patient's weight to predict the clearance. The formula, as originally published, is:

$$x = \frac{(140 - age) \times weight \times 0.85(if\,female)}{72 \times creatinine}$$

- This formula expects weight (actually mass) to be measured in Kg and creatinine to be measured in mg/dL, as is standard in the USA. The resulting value is multiplied by a constant of 0.85 if the patient is female. This formula is useful because the calculations are relatively simple.
- A modification of this formula, useful for the common units of measure, is:

$$x = \frac{(140 - age) \times weight \times constant}{creatinine}$$

- This formula uses metric units (weight in Kg, creatinine in µmol/L). The constant is 1.23 for men and 1.04 for women.

  It is usually expressed in ml per minute. Normal is 120 ml/min for anadult.

Note:

Usually, an adult will need dialysis because symptoms of kidney failure appear at a clearance of less than 10 ml/min. Creatinine clearance has to be measured by urine collection (usually 12 or 24 hours). It is a more precise estimate of kidney function than serum creatinine since it does not depend on the amount of muscle one has.

Lab Practices:

-Collect blood for serum preparation in an appropriate tube.

-Run Cr test and calculate CrC.

-Compare the results to the normal values.

Principal:

-Kit was purchased from spinreact.

The principal of this assay is based on the reaction of creatinine with sodium picrate as described by Jaffé.

Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents.

The intensity of the color formed is proportional to the creatinine concentration in the sample.

Reagents:

R1 **Picric Reagent**	**Picric acid**	**17.5 mmol/L**
R2 **Alkaline Reagent**	**Sodium hydroxide**	**0.29 mol/L**
Creatinine CAL	**Creatinine-aqueous primary standard**	**2 mg/dL**

Precautions:

R1(Picric acid): Corrosive (C):R35:Causes severe burns.

R2(NaOH): Irritant (Xi): R36/38: Irritating to eyes and skin.S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

S45: In case of accident or if you feel unwell, seek medical advices immediately.

Preparation:

Working reagent (WR):

Mix equal volumes of R 1 Picric Reagent and R 2 Alkaline reagent.

The working reagent is stable for 10 days at 15-25ºC.

Equipments:

- Spectrophotometer or colorimeter measuring at 492 nm (490-510).

- Matched cuvettes 1.0 cm light path.

- General laboratory equipment.

Samples:

- Serum or heparinized plasma.

Creatinine stability: 24 hours at 2-8ºC.

Procedure:

1. Assay conditions:

Wavelength: . . . . . 492 nm (490-510)

Cuvette: . . . . . 1 cm. light path

Temperature. . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water

3-Pipette into a cuvette:

	Blank	Standard	Sample
WR ml	1	1	1
Standard µl	----	100	----
Sample µl	----	----	100

4-Mix and start stopwatch.

5. Read the absorbance (A_1) after 30 seconds and after 90 seconds (A_2) of the sample addition.

6. Calculate: $\Delta A = A_2 - A_1$.

Calculation:

ΔA Sample - ΔA Blank / ΔA Standard - ΔA Blank X 2 (Standard conc.)= mg/dL of creatinine in the sample

Conversion factor: mg/dL x 88.4 = µmol/L.

Normal levels:

Male: o.7-1.4 mg/dl (61.8-123.7 µmol/L)

Female: 0.6-1.1 mg/dl (53.0-97.0 µmol/L)

Linearity:

From detection limit of 0.09 mg/dL to linearity limit of 15 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

References:

1. Murray R.L. Creatinine. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.

2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.

3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.

5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Lab Report

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Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

DESCRIPTION	RESULT	UNIT	REFERNCE RANGE
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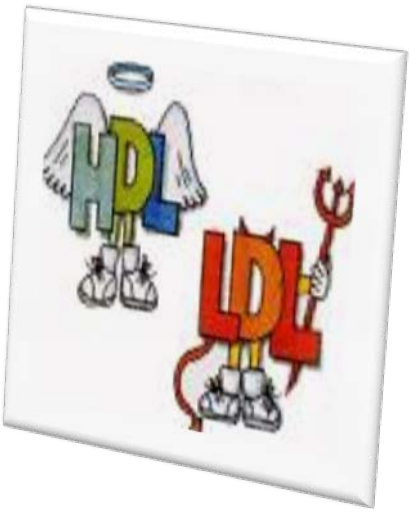
LAB-7

Lipid Profile

Measurement of Serum T- Chol, LDL-C, HDL-C and TG

What is lipid profile?



Lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. It includes total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG).

1- LDL-C (BAD CHOLESTEROL):

- It is type of lipoprotein that carries cholesterol in the blood.
- It is considered undesirable because it deposits excess cholesterol in tissues and organs as well as walls of blood vessel and contributes to hardening of the arteries and heart disease.
- Of all the forms of cholesterol in the blood, the LDL-C is considered the most important form in determining risk of heart diseases.

LDL values:

- LDL less than 100 mg/dL if you have heart disease or diabetes.
- LDL less than 130 mg/dL if you have 2 or more risk factors.
- LDL less than 160 mg/dL if you have 0 or 1 risk factor.

How LDL-C level is measured?

1- Direct LDL-C (by using method that measures it directly).

2- Calculated LDL-C (by using equation)

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

LDL = Total cholesterol – (HDL + TG/5)

2- HDL-C (GOOD CHOLESTEROL):

- It is type of lipoprotein that carries cholesterol in the blood.
- It is considered to be beneficial because it removes excess cholesterol from tissues.

HDL values:

- HDL less than 40 mg/dL ..... high risk of heart disease.
- A good level of HDL is ≥ 60 mg/dL.

3- Cholesterol:

- Cholesterol is a substance (a steroid) that is essential for life. It forms the membranes for cells in all organs and tissues in the body. It is used to make hormones that are essential for development, growth and reproduction. It forms acids that are needed to absorb nutrients from food. People consume it by eating animal products such as meat, eggs and dairy products. A small amount of the body's cholesterol circulates in the blood in complex particles called lipoproteins.
- Cholesterol is different from most tests in that it is not used to diagnose or monitor a disease but is used to estimate risk of developing a disease — specifically heart disease.

Cholesterol levels:

- Desirable: Cholesterol below 200 mg/dL is considered desirable and reflects a low risk of heart disease.
- High Risk: Cholesterol above 240 mg/dL is considered high risk. The doctor may order a lipid profile (as well as other tests) to try to determine the cause of high cholesterol.

Important note:

1-It is not necessary to fast when you have a cholesterol test. Cholesterol does not change in response to a single meal. Cholesterol does change in response to changes in long term patterns of eating — like changing from a high fat diet to a low fat diet — but it takes several weeks to see changes in blood cholesterol in response to changes in diet.

2-Cholesterol is high during pregnancy. Women should wait at least six weeks after the baby is born to have cholesterol measured.

3-Some drugs that are known to increase cholesterol levels include anabolic steroids, beta blockers, epinephrine, oral contraceptives and vitamin D.

4-Triglyceride(TG):

This test measures the amount of triglycerides in the blood. Triglycerides are the body's storage form for fat. Most triglycerides are found in adipose (fat) tissue. Some triglycerides circulate in the blood to provide fuel for muscles to work. Extra triglycerides are found in the blood after eating a meal—when fat is being sent from the gut to adipose tissue for storage. The test for triglycerides should be done when you are fasting and no extra triglycerides from a recent meal are present.

What does the test result mean?

A normal level for fasting triglycerides is less than 150 mg/dL. It is unusual to have high triglycerides without also having high cholesterol. When triglycerides are very high (greater than 1000 mg/dL), there is a risk of developing pancreatitis. Treatment to lower triglycerides should be started as soon as possible.

Specimen type, collection and storage:

Serum or plasma can be used. A fasting blood sample is preferred for lipid profile test. However, if cholesterol alone has to be analyzed, a random sample can also be used.

The specimen is stable for a week at 2 - 80C and at least for 3 months at -200C.

Lab practices:

-Collect blood for serum preparation in an appropriate tube.

-Run lipid profile tests.

-Compare the results to the normal values.

Principal (Cholesterol):

-The kit was purchased from spinreact.
-The cholesterol present in the sample originates a coloured complex, according to the following reactions:

Cholesterol esters + H_2OCholesterol + fatty acids ⟶–CHE

Cholesterol + O_2 ⟶–CHOD 4-Cholestenona + H_2O_2

2 H_2O_2 + Phenol + 4-AP Quinonimine + 4H⟶–POD$_2$O

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Principal (Triglyceride):

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2).

In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

Triglycerides + H_2OGlycerol + free fatty acids ⟶–LPL

Glycerol + ATP G3P+ ADP → kinaseGlycerol

G3P + O_2 DAP + H → PO_2O_2

H_2O_2 + 4-AP + p-Chlorophenol Quinone + H → POD_2O

The intensity of the color formed is proportional to the triglycerides concentration in the sample.

Samples:

Serum: Stability of the sample 7 days at 2-8ºC or freezing at –20ºC will keep samples stable for 3 months.

Procedure:

1. Assay conditions:

Wavelength: . . . . . 505 nm (500-550)

Cuvette: . . . . . 1 cm light path

Temperature . . . . . .37ºC /15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	standard	Sample
R ml	1	1	1
Standard µl	----	10	-----
Sample µl	----	-----	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 60 minutes.

Calculations:

A(sample /A(standard)x 200 (Standard conc.) = mg/dL cholesterol in the sample

Conversion factor (cholesterol): mg/dL x 0.0258= mmol/L.

Conversion factor (TG): mg/dL x 0.0113= mmol/L.

Reference values:

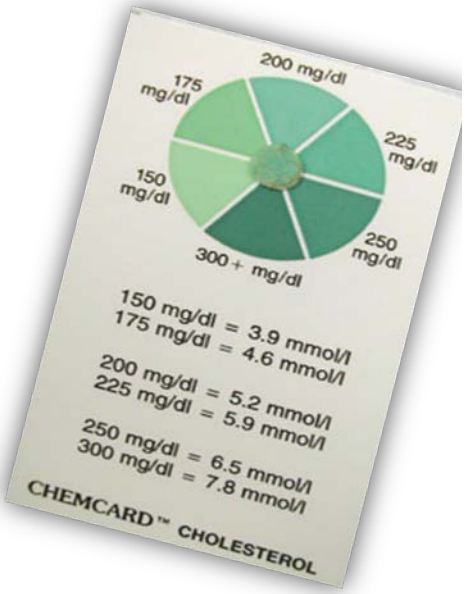
I-Cholesterol:

Risk evaluation:

Norma----------less than 200 mg/dl

Borderline-------200-239 mg/dl

High risk----------higher than 240 mg/dl



II-Triglyceride:

Men----------40-160 mg/dl

Women--------------35-135mg/dl

Measuring range:

From detection limit 0.46 to linearity limit 600 mg/dL. (Chol)

From detection limit 0.7 to linearity limit 1000 mg/dL. (TG)

If the concentration is greater than linearity limit dilute 1/2 the sample with NaCl 9 g/L and multiply the result by 2.

References:

1. Naito H.K. Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1194-11206 and 437.

2. Meiattini F. et al. The 4-hydroxybenzoate/4-aminophenazone Chromogenic System. Clin Chem 1978; 24 (12): 2161-2165.

1. Buccolo G et al. Quantitative determination of serum triglycerides by use of enzimes. Clin Chem 1973; 19 (5): 476-482.

2. Fossati P et al. Clin. Chem 1982; 28(10): 2077-2080.

3. Kaplan A et al. Tryglycerides. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 437 and Lipids 1194-1206.

Lab Report

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Patient Name:	**Age / Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

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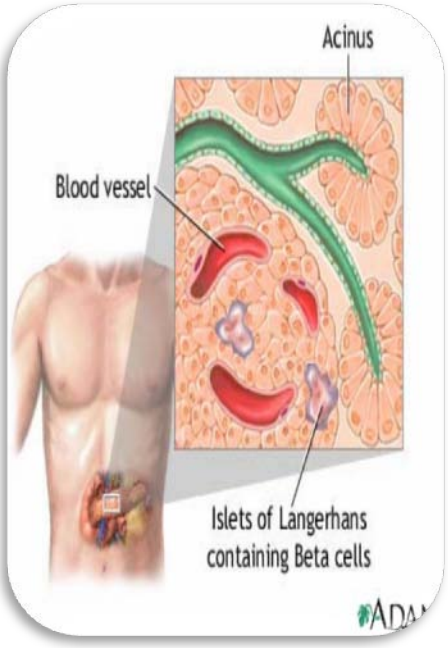
LAB-8

Diabetic Profile

Measurement of Blood Glucose

Diabetes mellitus is a group of metabolic disorders that requires continuing medical care and patient-self management education to prevent acute complications and to reduce the risk of long term complications.
- It is characterized by hyperglycemia and abnormal protein, fat and carbohydrate metabolism due to defects in insulin secretion, i.e., inadequate and deficient insulin action on target tissues.



It is classified into 4 clinical classes:

Type I diabetes mellitus (TIDM)

Type 2 diabetes mellitus (TIIDM)

Gestational diabetes mellitus (GDM)

Other specific types due to other causes e.g. Drugs or chemical induced.

Diabetic Profile Tests:

Group of tests that are used to diagnose diabetes and to measure response to treatment. They include:

1-C-peptide…..Differentiates between type I and type II.

2-Blood Glucose

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

- FBG (= FBS= PG=FPG)….Fasting blood glucose.
- PP glucose = Post prandial.
- GGT= Glucose tolerance test, OGGT= Oral glucose tolerance test

3-HbA1c = Glycosylated hemoglobin.
4-Ketones.
5-Microalbuminurea.
6-Insulin.
7-ICA = islet cell antibodies.

1- C-peptide:

Measurement of C-peptide exhibits a number of advantages over insulin measurement.

- It is better indicator of B-cell function than peripheral insulin.
- It doesn't measure the exogenous insulin.
- A C-peptide test can be done when diabetes has just been found and it is not clear whether type 1 diabetes or type 2 diabetes is present. A person whose pancreas does not make any insulin (type 1 diabetes) has a low level of insulin and C-peptide. A person with type 2 diabetes has a normal or high level of C-peptide.

Normal value: Fasting …..0.51-2.72ng/ml

Blood glucose:

- It is a vital component of diabetes management.
- In most cases, significantly elevated fasting glucose levels (>140mg/dl or > 7.77 mmol/l) are, in themselves, usually diagnostic of diabetes. However mild or borderline cases may present with normal FBG values. If diabetes is suspected, GTT can confirm the diagnosis.
- Occasionally, other diseases may produce elevated plasma glucose levels, therefore, a comprehensive history, physical examination and other tests should be carried out to confirm the diagnosis

Types of blood glucose tests:

(1)Fasting blood sugar (FBS) measures blood glucose after fasting for at least 8 hours. It often is the first test done to check for diabetes.

(2)2-hour postprandial blood sugar (2-hour PP) measures blood glucose exactly 2 hours after eating a meal.

(3)Random blood sugar (RBS) measures blood glucose regardless of when the person last ate. Several random measurements may be taken throughout the day. Random testing is useful because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may indicate a problem. This test is also called a casual blood glucose test.

(4)Oral glucose tolerance test (OGTT) measures the body's ability to use glucose. It is used mainly to diagnose prediabetes and diabetes. An oral glucose tolerance test is a series of blood glucose measurements taken after you drink a sweet liquid that contains glucose. This test is commonly used to diagnose diabetes that occurs during pregnancy (gestational diabetes). This test is not commonly used to diagnose diabetes in a person.

How to Prepare?

Fasting blood sugar (FBS)

For a fasting blood sugar test, the person should not eat or drink anything other than water for at least 8 hours before the blood sample is taken.

Random blood sugar (RBS)

No special preparation is required before having a random blood sugar test.

2-hour postprandial blood sugar (2-hour PP)

For a 2-hour postprandial test, the subject should eat a meal exactly 2 hours before the blood sample is taken. A home blood sugar test is the most common way to check 2-hour postprandial blood sugar levels.

Oral Glucose tolerance test (OGTT)

- On the day of testing, the following steps will be done:

- A blood sample will be collected when the subject arrives. This is the fasting blood glucose value. It provides a baseline for comparing other glucose values.
- Then a sweet liquid containing a measured amount of glucose will be given to the subject. It is best to drink the liquid quickly. For the standard glucose tolerance test, the subject will drink 75 g to 100 g; pregnant women drink 100 g of glucose.
- Blood samples will be collected at timed intervals of 1, 1.5 2, 2.5 and 3 hours after drinking the glucose.

Oral Glucose Tolerance Test
No food or drink 8 to 12 hours prior to test
Drink glucose
Blood is tested two hours later
High glucose level = potential diabetes
A.D.A.M.

- The oral glucose tolerance test is not commonly used to diagnose diabetes in people who are not pregnant. Many experts recommend using an oral glucose tolerance test if the result of a fasting blood glucose test is between 100 mg/dL (5.5 mmol/L) and 126 mg/dL (7.0 mmol/L).
- Glucose tolerance test screening by age 30 is recommend for all women who have polycystic ovary syndrome.

Glycosylated hemoglobin HbA1c:

- Glycosylated hemoglobin is an indicator of the blood glucose concentration over a longer period of time than either a single blood

glucose measurement (which reflects the glucose concentration at the time of blood collection)

- A glycohemoglobin test indicates how well diabetes has been controlled in the 2 to 3 months before the test.
- The A1C level is directly related to complications from diabetes: (The lower the A1C level, the lower the risk for complications)
- Normal values vary from lab to lab, depending on the test method used.

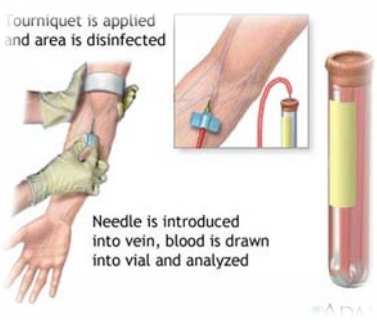
Normal Values:

Glycohemoglobin A1c:4.5%-5.7%

Total glycohemoglobin:5.3%-7.5%

Sample collection:

*W*hen whole blood is drawn, allowed to clot and kept centrifuged at room temp, the average decrease in serum glucose is approximately 7% in 1 hour. It is due to glycolysis by RBCs. The rate of glycolysis after 2 hours increases considerably, so the decrease in serum glucose may be about 50% or more.



- In separated non-hemolyzd serum, the glucose conc. is generally stable for 8 hours at 25C (or up to 72 hours at 4C)

- Glycolysis can be prevented by collecting blood in fluoride tube.

-For HBA1c EDTA tube (whole blood) is used

Lab Practices:

-Collect blood for FBS in an appropriate tube.

-Follow the method in the pamphlet.

-Compare the results to the normal value.

Principal:

-Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid.

-The formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD):

β-D-Glucose + O_2 + H_2O Gluconic acid + H——→–GOD$_2O_2$

H_2O_2 + Phenol + Aminophenazone Quinone + H——→–POD$_2$O

The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedure:

1. Assay conditions:

Wavelength: . . . . . 505 nm (490-550)

Cuvette: . . . . . 1 cm light path

Temperature. . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette

	Blank	Standard	Sample
WR ml	1	1	1
Standard µl	-----	10	-----
Sample µl	-----	-----	10

4. Mix and incubate for 10 min at 37ºC or 15-20 min at room temperature (15-25ºC).

5. Read the absorbance (A) of the samples and standard, against the Blank. The colour is stable for at least 30 minutes.

Calculations:

A(Sample)/A(Standard)x 100 (Standard conc.) = mg/dL glucose in the sample

Conversion factor: mg/dL x 0.0555= mmol/L.

Normal values:

Serum or plasma ------60-110mg/dl (3.33-6.10mmol/L)

Linearity:

From detection limit of 0.04 mg/dL to linearity limit of 500 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

References:

1. Kaplan L.A. Glucose. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1032-1036.
2. Trinder P. Ann Clin Biochem 1969; 6: 24-33.
3. www.webmed.com.

Lab Report

King Abdul-Aziz University
College of Science
Biochemistry Department
Clinical Biochemistry Course

Patient Name:	**Age / Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

BIOCHEMISTRY REPORT

DESCRIPTION	RESULT	UNIT	REFERNCE RANGE
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EXAMINED BY:
DATE/ TIME:

LAB-9

Routine Urine Analysis

Identification of Normal Physical and Chemical Urine Constituents

Urine:

- Is an ultrafiltrate of plasma from which glucose, amino acids, water and other substances essential to body metabolism have been reabsorbed.
- The physiological processes by which approximately 170000 ml of filtered plasma is converted to the average daily urine output of 1200 ml is complex.- Urine carries waste products, excess water or chemical substances from the body.
- In general, urine consists of urea (2%) and other organic and inorganic chemicals (2%) dissolved in water (96%).
- The concentration of these substances can be affected by various factors such as dietary intake, physical activity, body metabolism, endocrine function and others.
- Urea accounts for half of the total dissolved solids in urine. Other organic substances include primary creatinine and uric acid (UA).
- The major inorganic solid dissolved in urine is chloride, followed by Na and K.
- Small or trace amounts of many additional inorganic chemicals (sulfate, carbonate, etc.) are also present in urine.

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

Urine Analysis:

Urinalysis (UA) is an essential procedure for patients undergoing physical examination.

This is because: 1- Urine is readily available and easily collected sample.

2- Urine contains information about many of the body's ma metabolic functions, and this information can be obtained b simple laboratory tests.

Routine Urinalysis (Routine-UA):

- It consists of a group of screening tests generally performed as part of physical examination. It involves both macroscopic and microscopic analysis.

- It includes assessment of physical characteristics, chemical analysis (this is called macroscopic analysis) and microscopic examination of the (random) urine sample.

- First, the physical characteristics of the urine are noted and recorded. Second, a series of chemical tests is run. Third, the urine sediment is examined under the microscope to identify the components of the urinary sediments.

The purposes of performing a routine urine analysis are:

1. To aid in the diagnosis of disease
2. To screen for asymptomatic, congenital, or hereditary diseases
3. To monitor disease progression

4. To monitor therapy effectiveness or complications

Urine Collection:

Types of urine specimens:

The type of specimen selected and collection procedure used are determined by the health care provider and depend on the tests to be performed.

There are basically four types of urine specimens:

1. First morning specimen
2. Random urine specimen
3. Fractional collection
4. Timed collection

- During the course of 24h, the composition and concentration of urine changes continuously.

- Urine concentrations vary according to water intake and physical activities.

- To obtain a sample that is truly representative of patient's metabolic state, it is often necessary to regulate certain aspects of sample collection, such as time of collection, length of collection period, patient's dietary and medical intake and method of collection.

- Initial morning sample is preferred, particularly for protein analysis, because they are more concentrated from overnight retention in the bladder.

- The sample should be collected in a clean container.

- It must be analyzed within 1h of collection if held at room temp. or else refrigerated at $2^{o}C$ to $8^{o}C$ for not more than 8 hours before analysis.

- If not assayed within these time limits, several changes will occur.

- The urine container must be sterile if the urine is to be cultured.

- For microscopic examination, the urine must be fresh.

Urine strip:

The most cost-effective device used to screen urine is a paper or plastic dipstick. This microchemistry system has been available for many years and allows qualitative and semi-quantitative analysis within one minute by simple but careful observation. The color change occurring on each segment of the strip is compared to a color chart to obtain results.

I- Physical Characteristics:

-The first part of a urinalysis is direct visual observation. Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.

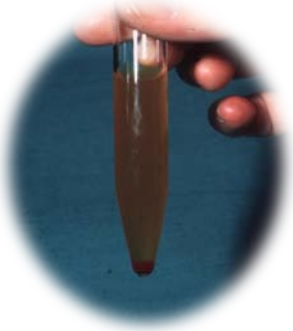
Examine the urine carefully by eye and ... nose! Comment on the color of the urine. Try to use words like yellow, amber, dark and pale. Examine the sample for its odor (smell). Also note whether the sample is clear or cloudy.

1- Colour:

- Colour intensity of urine correlates with concentration, the darker the colour, the more concentrated is the sample.

1- Amber yellow---------> Urochrome (derivative urobilin, the end product of bilirubin degradation a pigment found in normal urine.

2- Colourless-------> Reduced concentration.



3- Silvery sheen or milky appearance---------> Pus, bacteria or epithelial cells

4- Reddish brown----------> Blood (Hemoglobin).

5- Yellow foam---------> Bile or medications.

6- Orange, green, blue or red----------> medications.

2- Transparency:

- This is classified as clear and turbid.
- The degree of cloudiness of urine depends on both its pH and its dissolved solids composition.
- Turbidity may be due to gross bacteriuria, whereas smoky appearance is seen in hematouria.
- Threadlike cloudiness is observed when the sample is full of mucus.
- In normal urine, the main cause of cloudiness is crystals and epithelial cells.
- In pathological urine, it is due to pus, blood and bacteria.

3- Odour:

- Odour ordinarily has a little diagnostic significance.

1- Aromatic odour------> Normal urine due to aromatic acids.

2- Ammonical odour---------> On standing due to decomposition of urea.

3- Fruity odour--------> Diabetes due to the presence of ketones.

4- Volume:

- Urine volume measurements are part of the assessment for fluid balance and kidney functions.

- Most adults produce from 750ml-2500ml / 24h, with the average of about 1.5L per person.

- For RUA, a 10ml-12ml of sample is optimal for accurate of analysis.

5- Reaction (pH):

- The pH is a measure of how acidic or alkaline (basic) the urine is.

- Normal urine pH falls within the range of 4.5-8.

- Increased acidity in urine--------> Diabetes mellitus and some medications.

- The urine must be fresh (why?), owing to the marked tendency of urine to be alkaline on standing as a result of ammonia liberation.

6- Specific Gravity (SG):

-This measures the amount of substances dissolved in the urine. It also indicates how well the kidneys are able to adjust the amount of water in urine. The higher the specific gravity, the more solid material is dissolved in the urine

Normal Chemical Constituents of Urine:

1- Urea:

- 1ml urine + 3ml NaOCL (sodium hypochlorite) ========>Evolution of N_2 gas.

2- URIC ACID (UA):

- 1ml urine + 0.5 ml 10% NaOH +1ml Folin`s reagent ======> Blue colour.

3- Creatinine:

- 1ml urine + drops of sat. Picric acid + drops of NaOH 10%

=========> deep red colour of creatinine ppt.

- On acidifying with HCL, the colour changes to yellow.

4- Chloride:

- 1ml urine + drops dil. HNO_3 add 1 ml $AgNO_3$=====> white ppt of AgCL is formed which can be dissolved in NH_4OH

$$CL^- + AgNO_3 ==========> AgCL + NO_3$$

5- Phosphate:

- 1ml urine + 1ml conc. HNO_3 + 1ml ammonium molybdate=====>Yellow colour.

6- Carbonate:

- 1ml urine + drops conc. HCL ========> Effervescence

$$Na_2CO_3 + 2\ HCL ==========> H_2O + 2NaCL + CO_2$$

7- Ammonia:

- Make the urine just alkaline with NaOH. Close the tube with a cork containing another side tube dipped in Nessler's reagent. Heat the urine and then notice the evolving of NH_3 in Nessler's reagent – Detect NH_3 by its odour.

- 1ml urine + 1ml phenol + 1ml NaBr =======> Blue color.

8- Sulphates:

- 1ml urine + 2 drops conc. HCL + few drops $BaCL_2$ =======> White ppt of $BaSO_4$.

$SO_4 + BaCL_2 =====> BaSO_4 + 2CL^-$

Lab Practices:

- Collect urine in a clean container.
- Run routine UA on the sample by using both urine strip and the method described before for chemical analysis.
- Record the results in the lab report of UA.

References:

www.wekipidia.org

www.webmd.com

Fischbach, F., A Manual of Laboratory diagnostic Tests, Philadelphia, Lippincott, 1998.

Lab report

King Abdul-Aziz University
College of Science
Biochemistry Department

Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

URINE ANALYSIS

MACROSCOPIC EXAMINATION:

Amount			
Color			
Aspect			
SG			
pH			
Nitrite			
Protein			
Glucose			
Ketones			
Urobilinogen			
Bilirubin			
Blood			
Leucocytes			

EXAMINED BY:
DATE/ TIME:

LAB-10

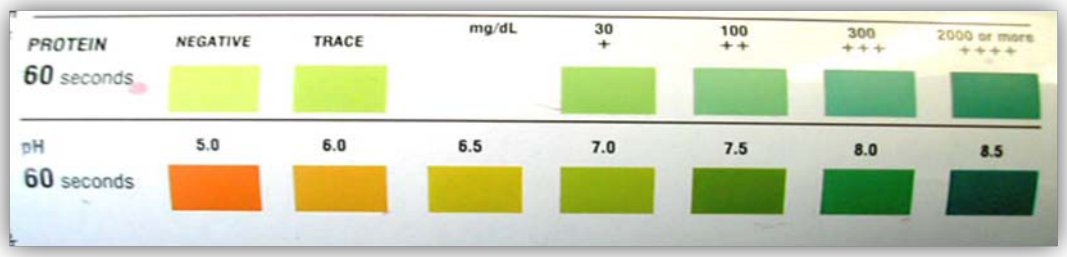
Routine Urine Analysis

Identification of Pathological Physical and Chemical Urine Constituents

Abnormal (Pathological)constituents of urine:

In determining whether pathological conditions exist through urine analysis, it is necessary to perform both physical and chemical tests. Of the physical tests that are available, only appearance of the urine will be observed. The chemical tests will be for pH, protein, glucose, ketones, hemoglobin. The significance of each abnormality will accompany the specific test. For detecting the presence of abnormal constituents, urine reagent strip will be used.

- Reagent strips provide a means of rapidly screening large numbers of urine specimens in a timely fashion for pH, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase. In addition, SG and ascorbic acid may also be screened.
- A reagent strip is an inert plastic strip onto which reagent-impregnated test pads are bonded. Chemical reactions take place when the strip is wetted in a urine sample. These reactions result in a color change that can be visually or mechanically assessed. By comparing the color change observed to the color chart supplied by the strip manufacturer, the laboratorian can determine qualitative results for each entity.



Depending on the test performed, the results are reported as:

1. In concentration (mg/dl)
2. As small, moderate, or large
3. Using the plus system (1+, 2+, 3+, 4+)
4. As positive, negative, or normal

Procedure:

1. Shake up the sample of urine and dip the test portion of a test strip into the urine. Tap the strip against the edge of the urine container to remove excess urine.

2. Immediately compare the test area with the color chart on the bottle. Note that the color scale runs from (negative) to (++++).

3. Record your results on the Urinalysis Worksheet.

Abnormal urine constituents include:

Proteinuria:

- Proteinuria is a condition in which urine contains an abnormal amount of protein.
- The majority of proteins found in the urine arise from the blood.

- As blood passes through healthy kidneys, they filter the waste products out and leave in the things the body needs, like proteins. Most proteins are too big to pass through the kidneys' filters into the urine unless the kidneys are damaged. The main protein that is most likely to appear in urine is albumin. Proteins from the blood can escape into the urine when the filters of the kidney, called glomeruli, are damaged. Sometimes the term albuminuria is used when a urine test detects albumin specifically.

- Large amounts of protein in the urine may cause it to look foamy. Also, because the protein has left the body, the blood can no longer soak up enough fluid leading to swelling in the hands, feet, abdomen, or face. These are signs of very large protein loss.
- In a healthy renal and urinary tract system, the urine contains no protein or only traces amounts.
- Because albumin is filtered more readily than the globulins, it is usually abundant in pathological conditions. Therefore, the term albuminuria is ~~~= proteinuria.
- The qualitative tests for proteinuria are most commonly performed using a reagent test strip. These methods depend on the change in the response of an indicator dye in the presence of protein.
- Quantitative examination of urine protein requires considerable attention to the volume or time of urine collection because the concentration may vary with time and volume.
- Most assays are performed on urine sample of 12-24h.
- Reference Value (RV) for 24-h urine

  Male = 1-4 mg/dl

  Female = 3-10mg/dl

  Child = 1-10mg/dl
- Reference value – Qualitative ------→ Normal = Negative

Glucosuria:

- Glucosuria is a condition in which urine contains an abnormal amount of glucose.
- Glucose is present in glomerular filtrate and is reabsorbed by the proximal tubule.
- If the blood glucose level exceeds the reabsorption capacity of the tubules, glucose will appear in the urine.
- Tubular reabsorption of glucose is by active transport in response to the body's need to maintain an adequate concentration of glucose.
- The blood level at which tubular reabsorption stops is termed renal threshold. The renal threshold of glucose is around 160 mg/100 ml. Glucosuria indicates that blood concentrations of glucose exceed this amount and the kidneys are unable to accomplish 100% reabsorption of this carbohydrate.
- Glucosuria occurs in diabetes mellitus, which characterized by abnormal blood glucose level (hyperglycemia) and usually increased volume of urine. The urine may be light in color and have a high specific gravity.

Ketonuria:

- Ketonuria is a condition in which urine contains an abnormal amount of ketone bodies.
- Normal catabolism of fats produces carbon dioxide and water as final end products. When there is not an adequate amount of carbohydrate in the diet, or when there is a defect in carbohydrate metabolism, the body begins to utilize an increasing amount of fatty acids. When this increased fat metabolism reaches a certain point, fatty acid utilization becomes incomplete, and intermediary products of fat metabolism occur in the blood and urine. These intermediary substances are the three ketone bodies: acetoacetic acid (diacetic acid), acetone, and beta hydroxybutyric acid. The presence of these substances in urine is called ketonuria.

- Diabetes mellitus is the most common disorder in which ketonuria occurs. Progressive diabetic ketosis is the cause of diabetic acidosis, due to the increased concentration of ketoacids which can eventually lead to coma or death. It is for this reason that the detection of ketonuria in diabetics is of great significance.

Normal Values

A negative test result is normal. Results of the presence of acetone in the urine are usually listed as small, moderate or large with these corresponding values:

Small - < 20 mg/dL

Moderate - 30-40 mg/dL

Large - > 80 mg/dL

What abnormal results mean?

A positive test may indicate:

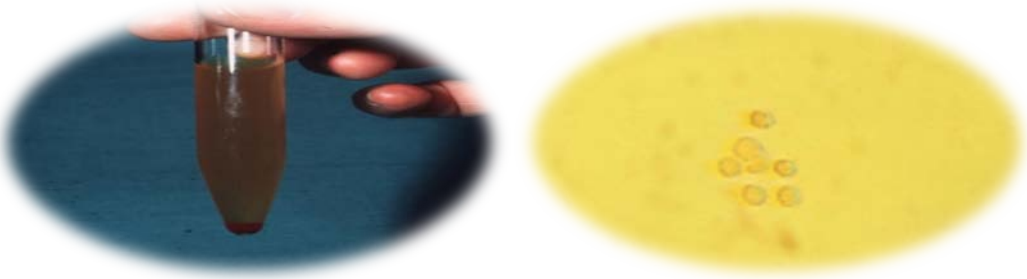
- Metabolic abnormalities, including uncontrolled diabetes or glycogen storage disease
- Abnormal nutritional conditions, including starvation, fasting, anorexia, high protein or low carbohydrate diets
- Vomiting frequently over a long period of time, including hyperemesis gravidarum (a severe form of morning sickness)
- Disorders of increased metabolism, including hyperthyroidism, fever, acute or severe illness, burns, pregnancy, lactation (nursing a baby), or post-surgical condition

Special considerations

- Special diets may alter test results. For example, a diet consisting of low amounts of carbohydrates with high amounts of protein and fat may affect the ketone levels.
- Drugs that may cause false positive measurements include glucocorticoids.
- In healthy persons, ketones are formed in liver and completely metabolized so that only negligible amounts appear in the urine.
- During pregnancy, the early detection of ketones is essential because ketoacidosis is a prominent factor that contributes to intrauterine death.

Hematuria and Hemoglobinuria:

- **Hematuria** is the presence of red blood cells (RBCs) in the urine. In microscopic hematuria, the urine appears normal to the naked eye, but examination under a microscope shows a high number of RBCs (see the figure). Gross hematuria can be seen with the naked eye—the urine is red or the color of cola.
- Several conditions can cause hematuria, most of them not serious. For example, exercise may cause hematuria that goes away in 24 hours. Many people have hematuria without any other related problems. Often no specific cause can be found. But because hematuria may be the result of a tumor or other serious problem, a doctor should be consulted.
- To find the cause of hematuria, or to rule out certain causes, the doctor may order a series of tests, including urinalysis, blood tests or others.
- **Hemoglobinuria** results from hemolysis i.e. the rupturing of erythrocyte and the liberation of hemoglobin. This may occur in malaria, typhoid, yellow fever, hemolytic jaundice and other diseases.



Bilirubin (Bile):

- Bilirubin is the waste product that results from the breakdown of hemoglobin molecules from worn out red blood cells. Ordinarily, it is excreted from the body as the chief component of bile. Excessive levels of bilirubin stain the fatty tissues in the skin yellow; this condition is called jaundice.

  Bilirubin is found in the dark urine of obstructive jaundice and later in the course of hepatitis. Urobilinogen is found in early hepatitis. It is colorless but darkens on standing. Bile pigments are included in the routine strip used in urinalysis. Positive results are an indication for measuring bilirubin and liver enzyme:

Leucocytes and nitrite test:

- **Urine leucocytes:** The esterase method is used to detect leucocytes in the urine by dipstick. The esterase released from the neutrophils reacts with the ester on the reagent strip releasing 3-0H-5-phenyl pyrrole that reacts with a diazonium salt to produce pink to purple colour. The test is usually positive if there are more than 5 leucocytes/hpf. Allowing the urine to stand promotes lysis of leucocytes and hence more intense reaction.
- False positives can occur with vaginal contamination. Presence of glucose, albumin, ascorbic acid, tetracycline, or large amounts of oxalic acid can inhibit the reaction.
- **Urine nitrite test** is another dipstick test used for screening for bacteria. Normal urine does not contain nitrites, but in the presence

of bacteria, the normally present nitrate in the urine is reduced to nitrite. This reacts with P-arsenilic acid, which further reacts with1,2,3,4 tetrahydrobenzoquinolin-3-ol giving a pink colour. It is a qualitative test: A positive test indicates presence of more than 10 organisms/ml.

Microscopic Examination:

Specimens should be examined macroscopically and microscopically. Urine sedimentation may contain cells, casts and crystals and is examined microscopically after centrifugation of a urine sample.

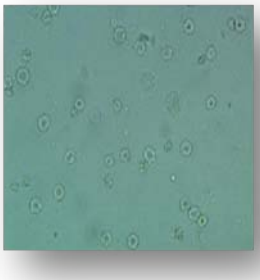
A very small amount of all of the above sediments is normal. Concern begins when any of these components is significantly elevated. There are many different crystals, cell types, and casts that may be found in the urine of animals. Listed below are some common findings in the urine of small animals.

-Crystals are common findings in urine especially if urine is allowed to stand long before examination. The type of crystal depends on the pH and constituents of the urine. Certain crystals like cystine, tyrosine, and leucine are always abnormal if detected. But other crystals are not abnormal, unless when associated with stone disease, in which case the type of crystal can give a clue to the origin of the stone. Exception is presence of large number of calcium oxalate crystals in association with ethylene glycol overdose.

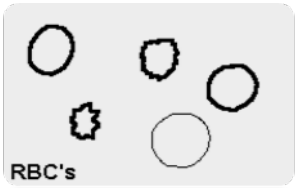
-Micro-organisms: Bacteria are the commonest organism seen and are commonly contaminants. The concomitant presence of WBC's however, suggests infection. Fungal elements can be seen, especially in females as contaminants. They may be pathogenic also. Apart from this, Trichomonas vaginalis, which is a protozoan, can be seen in women. Very rarely Schistosomia hematobium is seen in urine sediment.

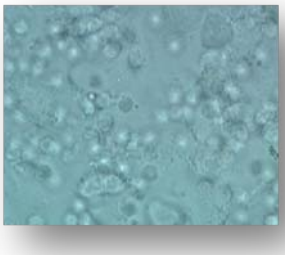
Procedure:

1. A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 r.p.m) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube.
2. The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube.
3. The sediment is resuspended in the remaining supernatant by flicking the bottom of the tube several times.
4. A drop of resuspended sediment is poured onto a glass slide and cover-slipped.
5. The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. Next, examination is carried out at high power to identify crystals, cells, and bacteria.

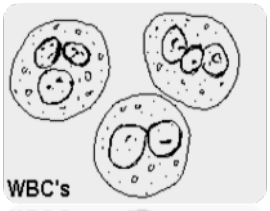


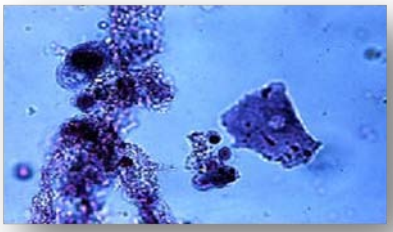
RBCs



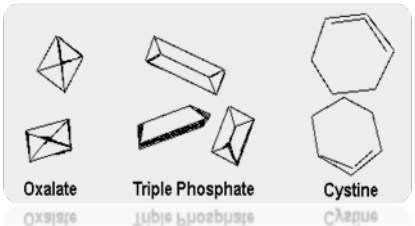


WBCs

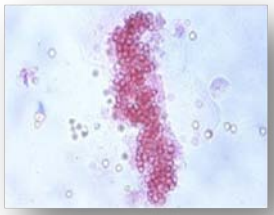




Epithelia cells



Blood



RBC cast

Ca oxalate crystal

Lab Practices:

- Collect urine in a clean container.
- Run routine UA and microscopic examination on the sample.
- Record the results in the lab report of urinalysis.

References:

1. Corwin HL. Urinalysis in Diseases of the Kidney (6th Ed) Ed : Schrier RW, Gottschalk, Boston Little Brown and Company. 1996; 1: 295-306.
2. Wise KA, Sagert LA, Grammens GL. Urine leucocyte esterase and nitrite tests as an aide to predict urine culture results. Lab Med 1984; 15: 186.
3. Guide to clinical preventive services, 2nd ed. Williams & Wilkins, Baltimore, 1996; pp 181-6.
4. Kasiske BL, Keane WF. Laboratory assessment of renal disease : Clearance, urinalysis and renal biopsy in the kidney (6th ed) Ed : Brenner BM, WB Saunders, Philadelphia 2000; pp 1142-53.
5. Norvin Peter AF. Urinary sediment in the interpretation of proteinuria. Annals of Internal Medicine 1983; 98: 254-5.
6. Schumann GB, Greenberg NF. Usefulness of macroscopic urinalysis as a screening procedure. A preliminary report. Am J Clin Pathol 1979; 71: 452-6.
7. Dinda AK. Supravital staining and bright field microscopy. A simple technique for urine sediment. The India J of Path And Micro 1999; 42 (3): 391-2.

Lab report

King Abdul-Aziz University
College of Science
Biochemistry Department

Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

URINE ANALYSIS

MACROSCOPIC EXAMINATION:

Amount			
Color			
Aspect			
SG			
pH			
Nitrite			
Protein			
Glucose			
Ketones			
Urobilinogen			
Bilirubin			
Blood			
Leucocytes			

MICROSCOPIC EXAMINATION:

EXAMINED BY:
DATE/ TIME:

LAB-11

Quantitative Determination of Urine Protein

Proteinuria & Microalbuminuria

Proteinuria:

From *protein* and *urine*- means the presence of an excess of serum proteins in the urine. The protein in the urine often causes the urine to become foamy although foamy urine may also be caused by bilirubin in the urine (bilirubinuria), pneumaturia (air bubbles in the urine).

Proteinuria is often diagnosed by a simple dipstick test although it is possible for the test to give a false negative even with nephrotic range proteinuria if the urine is dilute. False negatives may also occur if the protein in the urine is composed mainly globulins or Bence-Jones Proteins because the reagent on the test strips,Bromphenol blue, is highly specific for albumin. Anyone with foamy urine should be more thoroughly evaluated with more sensitive tests such as a 24-hour urine collection test even if the dipstick is negative for protein to completely rule out proteinuria.

Proteinuria may be a sign of renal (kidney) damage. Since serum proteins are readily reabsorbed from urine, the presence of excess protein indicates either an insufficiency of absorption or impaired filtration. Diabetics may suffer from damaged nephrons and develop proteinuria.

With severe proteinuria, general hypoproteinemia can develop which results in diminished oncotic pressure. Symptoms of diminished oncotic pressure may include edema.

Microalbuminuria

Is an increase in urinary albumin that is below the detectable range of the standard protein dipstick test. Microalbuminuria occurs long before clinical proteinuria becomes evident.

- It is the standard test for the screening, monitoring and detection of deteriorating renal function in diabetic patients.

الدراسات توضح أن مرضى السكر الذين يصلون لمرحلة الفشل الكلوي يتميزون بإفراز كميات بسيطة من البروتين على مدى سنوات وفي حال تم مراقبته وعلاجه بشكل جيد فإنه يمكن منع تطور المرض لحد الإصابة بالفشل الكلوي

When microalbuminuria is diagnosed?

- Normal < 30 mg / 24h

- Microalbuminuria 30–300mg of albumin/24 h
- or 20–200 µg/min or 30–300 µg/mg of creatinine on two of three urine collections
- Proteinuria > 300 mg / 24h

Significance:

- marker of vascular endothelial dysfunction
- an important prognostic marker for kidney disease
    - in diabetes mellitus
    - in hypertension
- increasing microalbuminuria level during the first 48 hours after admission to an intensive care unit predicts elevated risk for multiple organ failure , and overall mortality

Lab practices:

- Collect 24h urine sample.
- Determine the albumin level in this sample.
- Record the result in the lab report.

Principal:

Protein react in acid solution with pyrogallol red and molybdate to form a colored complex.

The intensity of the color formed is proportional to the protein concentration in the sample.

Reagents:

Pyrogallol red --------50mmol/L

Sodium molybdate------0.04mmol/L

Protein (standard)------1mg/L

Sample:

- Record the volume of 24h urine sample.
- Dilute the sample by 1:20

Procedure:

1. Assay conditions: Wavelength: . . . . . 598 nm.
   Cuvette . . . . . 1 cm light path
   Temperature 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Test
BCG ml	1	1	1
Standard µl	----	20	----
Sample µl	----	----	20

4. Mix and incubate for 10 min at room temperature (15-25ºC).

5. Read the absorbance (A) of the samples and Standard, against the Blank.

*The color is stable for 30 minutes at room temperature.

Calculations:

A (sample)/A (standard) x 1000 (Standard conc.) X Vol. (L) 24h urine= mg protein /24h.

Normal Values:

Urine-------<100mg/24h (<150mg/24h in pregnancy).

Linearity:

Up to linearity limit of 4000 mg/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

References:

1. Orsonneau JL et al. An improved Pyrogallol Red-Molybdate Method for Determining Total Urinary Protein. Clin Chem 1989 (35):2233-2236.

2. Koller A. Total serum protein. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1316-1324 and 418.

3. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.

4. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Lab report

King Abdul-Aziz University
College of Science
Biochemistry Department
Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

URINE ANALYSIS

MACROSCOPIC EXAMINATION:

Test	Concentration	NV
Protein /24h	----------	<100mg/24h
Albumin/24	----------	<30mg/24h

Comment:

EXAMINED BY:
DATE/ TIME:

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

LAB-12

Quantitative Determination of Urine Uric acid

Uric acid (UA): and its salt are the end products of purine metabolism.

- Purines such as adenosine and guanine from the breakdown of ingested nucleic acids or from tissue destruction are converted into UA mainly in the liver.
- UA is then transported by the plasma from the liver to the kidney, where it is filtered by glomerulus.
- In the urine at pH levels < 5.75, uric acid is the predominant form and frequently appears as uric acid crystals.

Why the test is performed?

The most common reason for measuring uric acid levels is for evaluation, diagnosis, and treatment of kidney stones. With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid.

Patients with gout may also be evaluated using this test, since a significant number of patients with gout develop uric acid kidney stones.

What abnormal results mean?

Abnormal results are indicated as follows:

Greater-than-normal urinary uric acid levels may indicate:

- Metastatic cancers
- Myelogenous and lymphoproliferative disorders
- High purine diet
- Gout

Lower-than-normal urinary uric acid levels may indicate:

- Chronic alcohol ingestion

- Chronic glomerulonephritis
- Lead poisoning

Lab practices:

- Collect 24h urine sample.
- Follow the instruction described in the pamphlet (kit was purchased from spinreact).
- Record the results in the lab report and compare with the normal values.

Principal:

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of POD, 4–aminophenazone (4-AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:

Uric acid + $2H_2O$ + O_2 Allantoine + CO $\rightarrow$ Uricase$_2$ + $2H_2O_2$

2H2O2 + 4-AP + DCPS Quinoneimine+ 4H2O $\rightarrow$ POD

The intensity of the red color formed is proportional to the uric acid concentration in the sample.

Sample:

- Urine (24 h): Stability 4 days at 15-25ºC, pH >8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor);

If urine is cloudy; warm the specimen to 60ºC for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

Procedure:

1. Assay conditions:

Wavelength: . . . . . .520 nm (490-550)

Cuvette: . . . . 1 cm light path

Temperature . . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Test
WR ml	1	1	1
Standard µl	-----	25	-----
Sample µl	-----	-----	25

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.

5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

Calculations:

Standard conc. = 6mg/dl

Urine 24 h

A (Sample) / A(Standard)x 6 x vol. (dL) urine 24 h =mg/24 h uric acid

Conversion factor: mg/dL x 59.5= µmol/L.

Linearity:

From detection limit of 0.03 mg/dL to linearity limit of 25 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Normal Values:

Urine-----250-270 mg/24h = 1.49-4.5 µmol/L

References:

BIBLIOGRAPHY

1. Schultz A. Uric acid. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.

2. Fossati P et al. Clin Chem 1980;26:227-231.

3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.

4. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Lab report

King Abdul-Aziz University
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Clinical Biochemistry Course

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

URINE ANALYSIS

MACROSCOPIC EXAMINATION:

Test	Concentration	NV
Uric Acid /24h	----------	250-270mg/dL/24h

Comment:

EXAMINED BY:
DATE/ TIME:

LAB-13

Quantitative Determination of Urine Creatinine& Creatinine Clearance

Creatinine Clearance:

As mentioned before in lab-6 CrC is the most precise method for determination of GFR because:

1- Cr is not absorbed by renal tubules.

2- The effect of fluid intake and excretion on CrC is much less than that of urea.

3- The blood Cr level is relatively stable.

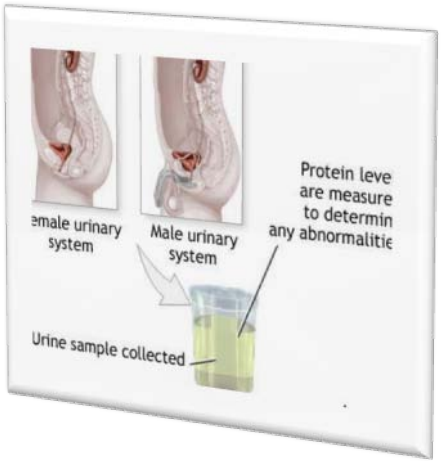
In RFTs lab CrC was measured by using equation but in clinical laboratories the most precise method for determining CrC is its measurement in 24h urine sample as well as in blood.

24h Urine collection:

-The patient should empty the bladder at the beginning of the period (8 a.m.) and discard the urine.

-Collect the urine passed until 8 o'clock the next morning , emptying the bladder at that time and adding this urine to the 24 urine sample

-The urine should be kept in cool place.


emale urinary system
Male urinary system
Protein leve are measure to determin any abnormalitie
Urine sample collected

Note: **-** Blood sample is collected for serum.

- Age and height are recorded.

Procedure:

1- Measure urine volume.

2- Determine serum (by the same method described in lab-6).

3- Determine urine creatinine.

Organized by: Lecturer: Sharifa A. Al-Ghamdi
Teaching Assistant: Ashwag A. Bukhari

Sample:

Urine: Dilute sample 1/50 with distilled water. Mix. Multiply results by 50 (dilution factor); Creatinine stability: 7 days at 2-8ºC.

Calculations:

$$CrC = \frac{\text{urine creatinine (mg/dl) X V (ml)}}{\text{Serum creatinine (mg/dl)}} \times \frac{1.73}{A}$$

1.73 = standard average surface area of the normal individual

A = Surface area of the patient

Procedure:

1. Assay conditions:

Wavelength: . . . . . 492 nm (490-510)

Cuvette: . . . . . 1 cm. light path

Temperature. . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water

3-Pipette into a cuvette:

	Blank	Standard	Sample
WR ml	1	1	1
Standard µl	----	100	----
Sample µl	----	----	100

4-Mix and start stopwatch.

5. Read the absorbance (A_1) after 30 seconds and after 90 seconds (A_2) of the sample addition.

6. Calculate: $\Delta A = A_2 - A_1$.

Calculation:

ΔA Sample - ΔA Blank / ΔA Standard - ΔA Blank X 2 (Standard conc.)= mg/dL of creatinine in the sample

Conversion factor: mg/dL x 88.4 = µmol/L.

Normal levels:

Male------10-20 mg/Kg/24h

Female------8-18 mg/Kg/24h

Linearity:

From detection limit of 0.09 mg/dL to linearity limit of 15 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

References:

1. Murray R.L. Creatinine. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.

2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.

3. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.

5. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

Lab report

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Biochemistry Department

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Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**
Doctor Name:	**Test Date:**

URINE ANALYSIS

MACROSCOPIC EXAMINATION:

Test	Concentration	NV
Creatinine /24h	----------	250-270mg/dL/24h
Serum creatinine	----------	0.6-1.1mg/dl
CrC	----------	8-18 mg/kg/24

Comment:

EXAMINED BY:
DATE/ TIME:

LAB-14

General View on CSF Analysis

Cerebrospinal Fluid (CSF):

Cerebrospinal fluid, CSF, is collected via the (LP) lumbar puncture. Most commonly today, specimen tubes are marked very clearly for the tests to be performed on that numbered specimen. The sterility of the specimen must also be maintained.

Cerebrospinal Fluid Examinations:

I-Appearance:

-Normal CSF is crystal clear, with the appearance and viscosity of water.

- Turbidity is graded from 1+ (slightly cloudy) to 4+ (very cloudy) and may be caused by the following conditions

1. :increased RBC's - red color
2. increased WBC's - cloudy
3. increased protein - cloudy
4. severe jaundice - slightly yellow
5. old blood - slightly yellow

The above abnormalities are easily detected, usually. The RBC's and WBC's may both be present in many inflammatory conditions. Appearance then, can be a good indicator of the type of problem present and can lay suspect certain pathological conditions.

II-Glucose:

CSF glucose levels are usually 1/2 of the serum glucose values (approx. 50-100 mg). The main pathologies occur when the CSF glucose is lower than normal. Decreased levels (45 mg and lower), are seen in meningitis, meningeal carcinoma and sometimes in intracranial hemorrhage.

III-Protein:

Normal levels of protein are 15-40 mg. Some disorders which can cause an increase in protein can also cause an increase in the WBC count as well. The following list of disorders can cause increased protein in the CSF; some also cause a corresponding elevation in WBC's:

1. brain tumors
2. some diabetics
3. multiple sclerosis
4. syphilis

IV- Cell Count:

As discussed earlier, many disorders can cause increased cell counts in the spinal fluid. The first two specimen containers obtained will be contaminated with blood cells due to the trauma of the Lumbar Puncture itself.

Therefore, the cell count is usually performed on the last of the specimens taken. This is one of the reasons for correctly marking the specimen tubes as they are obtained. Most of the new disposable Lumbar Puncture trays today have conveniently pre-marked specimen containers for each successive specimen. This reduces the risk of mismarking the containers.

A cell count is usually performed in order to detect the presence of infection. Upon examination, the lymphocytes are examined and their presence less than 500mm3 may indicate a viral infection, or over 500mm3 may indicate purulent infections (increased granulocytes). In

addition, a WBC differential count may be ordered so that the individual types of WBC's can be identified.

-Normally CSF is free of cells.

adult	**0-8 leukocytes per cubic mm (mm3)**
child	0-8 leukocytes per cu mm
newborn	0-15 leukocytes per cu mm
premature infant	0-20 leukocytes per cu mm

V-Culture:

This test usually is performed when meningitis or other infection is suspected. Usually a culture of the CSF is a routine procedure on all specimens collected.

VI-Serology:

This test for CSF serology can have great clinical significance. Many times when the blood serology test is negative, the CSF test is positive. An example of this is: tertiary syphilis; where the serum test turns negative with time. There are also other times when the CSF test will be negative and the serum test will be positive. Each case must be evaluated individually. If syphilis is suspected, a CSF serology may be done in the presence of negative blood serology report from the lab.

VII-Soluble Amyloid Beta Protein Precursor:

The presence of the amyloid beta protein in the senile plaques of the brain is a hallmark of Alzheimer's Disease, leading researchers to believe that this protein may be responsible for the disease's neurotoxic effects. Although amyloid is found in the CSF of healthy people, it is found in

smaller amounts in some patients with dementia, making it a useful diagnostic tool.

Findings:

Soluble amyloid beta protein precursor is found in the CSF of healthy people.

- Normal amyloid beta protein levels in CSF are greater than 450 units/L, based on age-matched controls using the ELISA test.
- Low CSF levels suggest an alteration in the amyloid beta protein precursor processing and amyloid beta protein formation. Low soluble amyloid beta protein precursor levels correlate with clinically diagnosed and autopsy-confirmed Alzheimer's disease.

References:

Knight, JA: Advances in the analysis of cerebral spinal fluid. Am Clin Lab Sci 27:93, 1997.

Smith S, Forman D: Laboratory analysis of cerebrospinal fluid. Clin Lab Sci 7(4):32-38, 1994.

Important Note:

Body Fluid Lab Tests:

The tests presented in this note are some of the most commonly used tests in most hospitals today. There are other special tests which can be performed on these fluids, blood, urine and spinal fluid, but usually just for rare conditions. There are also many other body fluids which may be tested. These include, but are not limited to:

synovial fluid, Pericardial fluid, pleural fluid, sweat, urogenital secretions, sputum, gastric acid, peritoneal fluid, fecal lipids, bile, semen, amniotic fluid, and many others…….

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List for laboratory investigations

Patient Name: **Age:** **years**

Patient ID: **Sex:** ○ **Male** ○ **Female**

Clinical History:

Diagnosis:

Date: / /
○ **Routine** ○ **State**
Investigation Required:

BLOOD BANK

☐ 1001 Whole Blood
☐ 1002 Anti-body Screening
☐ 1003 BL.GR + RH ABO
☐ 1004 Coomb's Test Direct
☐ 1005 Coomb's Test Indirect
☐ 1006 Cross Matching
☐ 1007 Fresh Frozen Plasma

HEAMTOLOGY / COAGULATION

☐ 2001 Acid Phosphatase Stain
☐ 2002 Activated Clotting Time
☐ 2003 Anti-Thrombin III
☐ 2004 Bleeding Time
☐ 2005 blood Film For Malaria
☐ 2006 Bone Marrow Examination
☐ 2007 CBC
☐ 2008 CBC with Differential
☐ 2009 Clotting Time
☐ 2010 Clotting Factor V
☐ 2011 Clotting Factor VII
☐ 2012 Clotting Factor VIII
☐ 2013 Clotting Factor IX
☐ 2014 Clotting Factor X
☐ 2015 Clotting Factor XI
☐ 2016 Clotting Factor XII
☐ 2017 ESR
☐ 2018 F.D.Ps
☐ 2019 Fibrinogen
☐ 2020 Hb
☐ 2021 HCT
☐ 2022 Iron Stain
☐ 2023 Osmotic Fragility Test
☐ 2024 Partial Thromboplastin Time
☐ 2025 Platelet Count
☐ 2026 Protein C
☐ 2027 Protein S
☐ 2028 Prothrombin Time (PT)
☐ 2029 Reticulocyte Count
☐ 2030 Sickling Test
☐ 2031 Sudan Black
☐ 2032 Thrombin Time
☐ 2033 D-Dimer

CLINICAL CHEMISTRY

☐ 3001 A.G. Ratio
☐ 3002 Acid phosphatase
☐ 3003 Albumin
☐ 3004 Albumin & Globulin
☐ 3005 Alkaline Phosphatase
☐ 3006 Ammonia
☐ 3007 Amylase
☐ 3007 Bicarbonate
☐ 3008 Bilirubin Direct & Total
☐ 3009 Blood Gases & Ph (ABG)
☐ 3010 C.K. (CPK)
☐ 3011 C.K.M.B.
☐ 3012 Calcium
☐ 3013 Carbon Dioxide
☐ 3014 Cholesterol
☐ 3015 Cholesterol (HDL)
☐ 3016 Cholesterol (LDL)
☐ 3017 CHOLINESTERASE
☐ 3018 Creatinine
☐ 3019 Creatinine Clearance
☐ 3020 CSF Glucose
☐ 3021 CSF Protein
☐ 3022 Electrolyte Chloride (Cl)
☐ 3023 Electrolyte Sodium (Na)
☐ 3024 Electrolyte Potassium (K)
☐ 3025 Protein Electrophoresis
☐ 3026 Hb Electrophoresis
☐ 3027 γ-GT (G.G.T.)
☐ 3028 G6PD (Quantitative)
☐ 3029 Glucose RBS
☐ 3030 Glucose FBS
☐ 3031 Glucose PPBS
☐ 3032 Glucose Total Tolerance (OGTT)
☐ 3033 Glycosalyted Hb
☐ 3034 Iron
☐ 3035 L.D.H.
☐ 3036 Lipase
☐ 3037 Magnesium
☐ 3038 Microalbuminuria
☐ 3039 Occult Blood in Stool
☐ 3040 Osmolality (Urine, blood)
☐ 3041 Phosphorus
☐ 3042 Pregnancy Test (urine)
☐ 3043 Pregnancy Test (Serum)
☐ 3044 SGOT
☐ 3045 SGPT
☐ 3046 Semen Fructose
☐ 3047 Stone Analysis
☐ 3048 Stool Analysis
☐ 3049 T.I.B.C
☐ 3050 Total Proteins
☐ 3051 Triglycerides
☐ 3052 Urea (BUN)
☐ 3053 Urea Clearance
☐ 3054 Uric Acid
☐ 3055 Uric Acid Clearance
☐ 3056 Urine Analysis
☐ 3057 Urine Albumin
☐ 3058 OGTT Screening
☐ 3059 OGTT Conformation
☐ 3060 Lactic Acid